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Signaling capacity of FcγRII isoforms in B-CLL cells

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Abstract

Two main isoforms of Fcγ receptor II (CD32) have been described in humans: activatory FcγRIIA and inhibitory FcγRIIB. We have previously reported that B cells from a subset of chronic lymphocytic leukemia (B-CLL) patients express not only FcγRIIB, as normal B lymphocytes, but also the myeloid FcγRIIA. The aim of this study was to evaluate the signaling capacity of both FcγRII isoforms in B-CLL cells. We found that FcγRIIA expressed by leukemic cells failed to induce Ca^{2+} mobilization or protein tyrosine phosphorylation, suggesting that the receptor is not functional. By contrast, FcγRIIB effectively diminished BCR-triggered ERK1 phosphorylation, which indicates that it is able to transduce inhibitory signals in B-CLL cells. Moreover, we found that FcγRIIB homoaggregation in either B-CLL or non-malignant tonsillar B cells did not result in apoptosis as was reported for murine B splenocytes. Together, these results show that FcγRIIB, but not FcγRIIA is biologically active in B-CLL cells and might influence leukemic cell physiology in vivo.

Keywords: FcγRIIA; FcγRIIB; B-CLL

1. Introduction

Human Fcγ receptor II (CD32) exists in two main isoforms, FcγRIIA and FcγRIIB, which are highly homologous in their extracellular and transmembrane regions but exhibit significant divergence in their cytoplasmic domains [1–3]. FcγRIIA is an activation receptor characterized by the presence of an immunoreceptor tyrosine-based activation motif (ITAM) sequence which becomes phosphorylated on tyrosines following binding of ligand at the cell surface [1–3]. On the other hand, FcγRIIB is an inhibitory FcγR that bears an immunoreceptor tyrosine-based inhibition motif (ITIM) sequence in its cytoplasmic domain. Coagreggation of ITAM-bearing receptors and FcγRIIB induces ITIM phosphorylation and (SH2)-containing inositol phosphatases (SHIP) recruitment which are responsible for its inhibitory activity [1,4,5]. In murine B cells, homoagreggation of FcγRIIB can also signal independently of ITIM phosphorylation generating a proapoptotic cascade through the activation of the Bruton tyrosine kinase (Btk) [6]. Signaling pathways triggered by FcγRIIA can also regulate cell survival as was demonstrated in human neutrophils by our group [7] and in eosinophils by others [8].

B-cell chronic lymphocytic leukemia (B-CLL) is characterized by the progressive accumulation of clonal $CD5^+$ B lymphocytes which seems to result from decreased apoptosis rather than increased proliferation [9–11]. We have previously reported that B-CLL cells display comparable or even

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higher levels of membrane FcγRIIB than normal B lymphocytes [12]. Interestingly, we have also observed that, unlike normal B cells, leukemic cells from a proportion of B-CLL patients express FcγRIIA [13], which is characteristic of myeloid leukocytes. Numerous signaling defects have been described in B-CLL. Thus, CD40-mediated signal transduction was severely impaired in all B-CLL patients [14], while responses through other cell surface receptors, such as BCR [15–17] or CD5 [18] are heterogeneous among CLL clones. Given that signals triggered through FcγRII are able to modify the survival and activation status of different cell types, including B lymphocytes, the aim of this study was to evaluate whether FcγRIIA and FcγRIIB are functional in B-CLL cells.

2. Materials and methods

2.1. Antibodies

Goat anti-mouse IgG Fab'_2 (GAM) FITC-conjugated were obtained from Immunotech (Marseille, France) and MoAbs IV.3 and AT.10 were kindly provided by Dr. Daeron (Curie Institute, Paris, France). Fab fragment of IV.3 were obtained by papain digestion as it was described by Harlow and Lane [19]. Purified goat anti-mouse IgG Fab'_2 (GAM), anti-IgM IgG and anti-IgM Fab'_2 fragments were obtained from Jackson ImmunoResearch (West Grove, PA, USA). Antibodies against phospho-tyrosine (mouse, clone 4G.10, Upstate), phospho-ERK1/2 (mouse IgG, Cell Signaling, Beverly, MA), actin (mouse IgM, Oncogene, Boston, MA) and HRP-conjugated anti-mouse IgG (BD Biosciences, San Diego, CA) were used for Western blot analysis.

2.2. B-CLL patients

Peripheral blood samples were obtained from 27 typical B-CLL patients (16 men and 11 women), who were informed about the objectives of the study and gave their consent. Patients enrolled had a median age of 69 years old and were classified by RAI system: 16 patients were RAI 0–I (indolent disease), five patients were RAI II (intermediate disease) and six were RAI III–IV (aggressive disease) (see Table 1). At the time of the analysis, all patients were free from clinically relevant infectious complications and were untreated or had received no treatment over the prior 6 months.

2.3. B-CLL cell isolation

Peripheral blood mononuclear cells from B-CLL patients (PBMC) were isolated by centrifugation over a Ficoll–Hypaque layer (Hystopaque), washed twice with saline and resuspended in complete medium (RPMI 1640, 10% FCS, 100 U/ml penicillin and 100 μg/ml streptomycin). B cell purification from PBMC were performed as previously described [20]. Briefly, PBMC were incubated with MoAbs specific for CD3, CD14, CD16 and CD56 during 45 min at 4 °C, washed twice and treated with magnetic beads coated with antimouse IgG antibodies (Dynabeads M450, Dynal, Oslo, Norway), according to the manufacturer's instructions. The purity of B-CLL population was checked by flow cytometry analysis using anti-CD19 MoAb and was found to be always >96%.

Table 1
Main clinical characteristics of enrolled B-CLL patients and FcγRIIA expression

Patient	Gender	Age	WBC, $\times 10^9$/l	LC, $\times 10^9$/l	RAI	FcγR IIA
1	M	89	23.1	21	0	+
2	M	64	9.1	5.5	0	–
3	F	65	36.5	23.7	0	–
4	M	66	37	29	0	+
5	F	77	25.3	20	0	–
6	M	60	56.6	53.7	0	–
7	M	63	6.3	5.8	0	–
8	F	78	77	73	0	+
9	M	75	35	27	0	–
10	M	74	14.7	11.3	0	+
11	M	76	67.1	63.7	0	+
12	M	84	41.3	33	I	+
13	F	52	15.2	11.2	I	–
14	M	67	32.1	29	I	–
15	M	66	51.4	43.7	I	–
16	M	58	40.9	32.7	I	+
17	F	74	27.6	23.6	II	–
18	M	61	208	206	II	–
19	M	62	18.7	12	II	++
20	F	73	38.6	34	II	+
21	M	56	19.7	15.6	II	++
22	F	75	50.1	45.3	III	–
23	F	62	43.7	42.5	III	+
24	M	76	64.6	61.3	III	+
25	F	74	76.8	72.8	III	+
26	F	70	50.4	48	IV	+
27	F	81	62.9	61	IV	++

The expression of FcγRIIA in B-CLL cells was evaluated by using Fab fragments of IV.3 MoAb (3 μg/ml) or isotype-matched Ab (3 μg/ml) and saturating concentrations of GAM FITC-conjugated. Data were analyzed by employing CellQuest software and the mean fluorescence intensity (MFI) of each sample was obtained. Arbitrary units of MFI from isotype controls were always lower than 10. As it is shown, patients were classified in three groups depending on the levels of FcγRIIA expression on $CD19^+$ cells: negative samples (MFI < 10) were depicted as '–'; positive samples with MFI between 10 and 50 were depicted as '+' and samples with MFI >50 were depicted as '++'. WBC indicates white blood cell counts and LC, lymphocytes counts.

2.4. Tonsillar B cell isolation

Tonsils were obtained from patients undergoing routine tonsillectomies for obstructed breathing disorders. Patients were informed about the objectives of the study and gave their consent. Tonsils were kept in cold isotonic saline and processed as previously described [21]. Briefly, tissue samples were cut and pushed through mesh using the flat end of a 60 ml

plastic syring plunger. Mononuclear cells from tonsils samples were isolated by centrifugation over a Ficoll–Hypaque layer and B cell purification were performed as described above. The purity of B cell population was checked by flow cytometry analysis using anti-CD19 MoAb and was found to be >96%.

2.5. Calcium mobilization

Changes in intracellular calcium mobilization (Ca^{2+}) induced by FcγRIIA crosslinking was evaluated in purified B-CLL cells and PBMC from the same patient by using FLUO-3AM (Sigma Chemical Co., St. Louis, MO, USA) and flow cytometry analysis, as previously described [7]. Briefly, cells were incubated with 4 mM FLUO-3AM for 30 min at 30 °C, washed twice and resuspended in complete medium. Aliquots of this cell suspension were then treated with IV.3 Fab for 15 min at room temperature, washed, resuspended in complete medium containing 1 mM $CaCl_2$ and warmed at 37 °C. The samples were immediately loaded onto the flow cytometer, and the basal fluorescence (FL1) was recorded during 90 s. Then, cells were stimulated with GAM in order to crosslink FcγRIIA, and the fluorescence was recorded during an additional 400 s. Acquisition of samples was performed at 37 °C and Ca^{2+} mobilization was recognized as an increase in FLUO-3AM fluorescence. Data were analyzed by employing CellQuest software (Becton Dickinson, Mountain View, CA). A gate based on forward and side scatters was used to evaluate monocytes Ca^{2+} mobilization in PBMC.

2.6. Western blotting

Time course experiments were carried out to evaluate FcγRII signaling capacity. Protein tyrosine phosphorylation induced by FcγRIIA crosslinking was evaluated by culturing cells (2×10^6) with or without IV.3 Fab ($n = 6$). Then, cells were washed, resuspended in complete medium containing GAM and incubated at 37 °C. FcγRIIB inhibition of BCR-induced ERK1/2 phosphorylation was evaluated by culturing B-CLL cells (2×10^6) at 37 °C with 15 μg/ml of anti-IgM Fab'_2 (F) or whole molecule (W) ($n = 6$). Reactions were stopped at the times indicated by adding cold saline and the samples were analyzed by Western blot. To this aim, cell pellets were washed with cold saline, immediately resuspended in loading buffer (60 mM Tris pH 6.8, 2.3% SDS, 10% glycerol, 0.01% bromophenol blue and 5% β-mercaptoethanol) and boiled at 96 °C for 5 min. Samples were then separated on a standard 10% SDS–PAGE and transferred to PVDF membranes (Sigma). Membranes were then blotted with antibodies against phospho-tyrosine, phospho-ERK1/2 or actin, followed by HRP-conjugated anti-mouse IgG. Specific bands were developed by enhanced chemiluminiscence (ECL, Amersham Pharmacia Biotech, Piscataway, NJ).

2.7. Quantitation of cellular apoptosis and viability by fluorescence microscopy

Quantitation was performed as previously described [22], using the fluorescent DNA-binding dyes acridine orange (100 μg/ml) to determine the percentage of cells that had undergone apoptosis, and ethidium bromide (100 μg/ml) to differentiate between viable and nonviable cells. With this method, nonapoptotic cell nuclei show variations in fluorescent intensity that reflect the distribution of euchromatin and heterochromatin. By contrast, apoptotic nuclei exhibit highly condensed chromatin that is uniformly stained by acridine orange. To assess the percentage of cells showing morphologic features of apoptosis, at least 200 cells were scored in each experiment.

2.8. Quantitation of cellular apoptosis by propidium iodide staining and flow cytometry

The proportion of cells that displays a hypodiploid DNA peak was determined using a modification of Nicoletti's protocol [23]. Briefly, cell pellets (2.5×10^6 cells) were suspended in 400 μl of hypotonic fluorochrome solution (propidium iodide, 50 μg/ml in 0.1% sodium citrate plus 0.1% Triton X-100) and incubated for 2 h at 4 °C. The red fluorescence of propidium iodide of individual nuclei was measured using a FACScan flow cytometer. The forward scatter and side scatter of particles were simultaneously measured. Cell debris was excluded from analysis by appropriately raising the forward scatter threshold.

2.9. Statistical analysis

Student's paired *t*-test was used to analyze the statistical significance of the experimental results.

3. Results

3.1. Functional analysis of FcγRIIA expressed on B-CLL cells

Clinical characteristics of B-CLL patients enrolled are summarized in Table 1. The expression of the myeloid FcγRIIA was determined by using the MoAb IV.3, which specifically recognizes FcγRIIA when used as Fab fragment [24–27], and goat anti-mouse IgG Fab'_2 (GAM) FITC-conjugated. We confirmed our previous data [13] showing that FcγRIIA is aberrantly expressed on leukemic cells from 15 out of 27 patients analyzed (Table 1), being the mean fluorescence intensity (MFI) generally low (arbitrary units of MFI < 50), while patients 19, 21 and 27 show higher expression. Representative histograms from three patients with different levels of FcγRIIA expression on $CD19^+$ cells are depicted in Fig. 1A. As expected, gated monocytes present in PBMC from the same patient displayed high levels of the

receptor (see inserts of Fig. 1). To further confirm that IV.3 (Fab) specifically recognizes FcγRIIA and not FcγRIIB, we also stained normal B cells. In accordance with previous reports [3–5], surface FcγRIIA was neither detected in tonsillar B cells (Fig. 1A) nor in circulating B cells from age matched normal donors (not shown).

The aggregation of FcγRIIA in myeloid cells triggers activation signals that result in tyrosine phosphorylation of different cellular proteins and Ca^{2+} mobilization [1–3]. In order to determine whether FcγRIIA present on B-CLL cells was able to induce cellular activation, we evaluated Ca^{2+} mobilization and protein tyrosine phosphorylation upon FcγRIIA crosslinking with IV.3 and goat anti-mouse IgG. Considering that B-CLL cells express high levels of FcγRIIB, Fab fragments of IV.3 and Fab'_2 fragments of goat anti-mouse IgG (GAM) were used to avoid the interaction of these antibodies through their Fc portion. Results in Fig. 1B show that homoaggregation of leukemic FcγRIIA did not trigger Ca^{2+} mobilization, while gated monocytes present in PBMC from the same patient displayed a significant response. Similar results were obtained after extensive crosslinking by addition of sheep anti-goat IgG Fab'_2 (not shown). Protein tyrosine phosphorylation upon FcγRIIA homoaggregation was evaluated by Western blot as described in Section 2. Time course experiments were carried out with purified B-CLL cells and PBMC from the same patient. As previously reported [17], we observed two proteins of aproximately 55 kDa which appear to be constitutively phosphorylated in B-CLL cells independently of anti-FcγRIIA stimulation (Fig. 1C). In all the samples analyzed, specific crosslinking of FcγRIIA failed to induce detectable protein tyrosine phosphorylation. This could not be attributed to technical pitfalls because PBMC from the same patient showed a clear response (Fig. 1C, right panel), most probably due to crosslinking of monocytic FcγRIIA. Given that monocytes comprised less than 3% of PBMC in all the samples analyzed, the method we used to homoaggregate FcγRIIA was highly efficient. Taken together, these results indicate that FcγRIIA expressed on B-CLL cells is unable to transduce activatory signals.

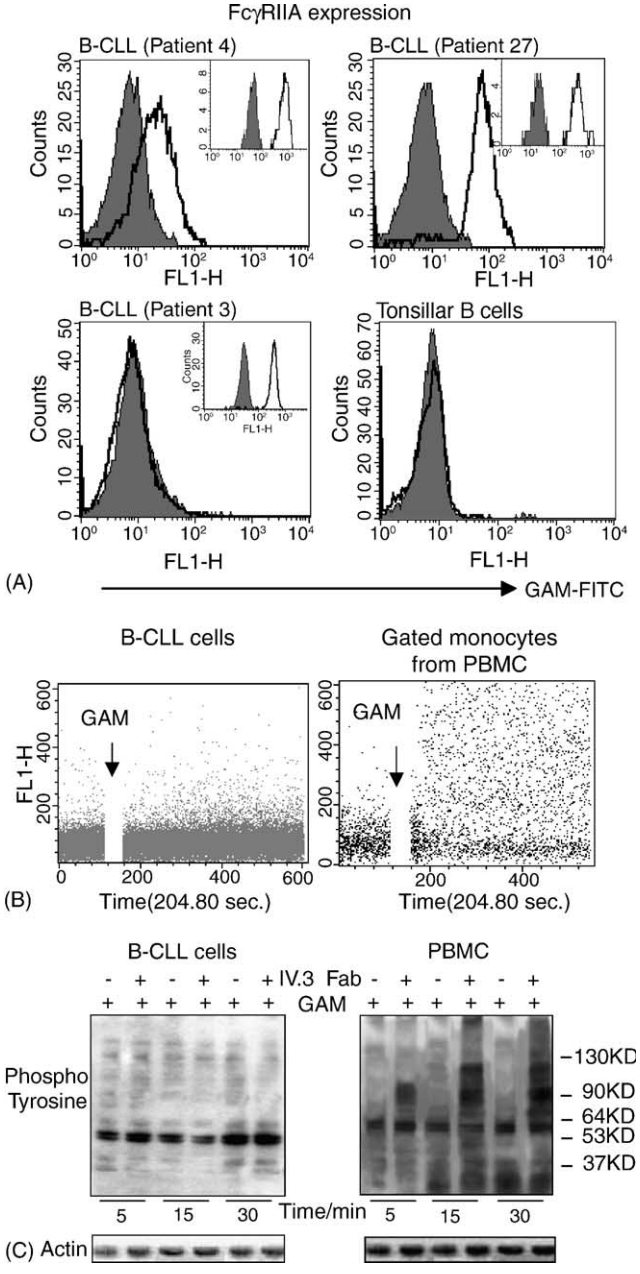

Fig. 1. Functional analysis of FcγRIIA. (A) The expression of FcγRIIA was evaluated by using Fab fragments of IV.3 MoAb (solid line) or isotype-matched Ab (grey area) and saturating concentrations of GAM FITC-conjugated. The figure shows FcγRIIA expression on leukemic cells ($CD19^+$) and gated monocytes (see inserts) from three representative patients. The FcγRIIA expression on non-leukemic tonsillar B cells is also shown. (B) Ca^{2+} mobilization induced by FcγRIIA crosslinking was evaluated in purified B-CLL cells and peripheral blood mononuclear cells (PBMC) from the same patient by flow cytometry. Cells previously loaded with FLUO-3AM, were treated with IV.3 Fab and FcγRIIA was crosslinked by adding GAM (arrow). Ca^{2+} mobilization was recognized as an increase in FLUO-3AM fluorescence. Results from a representative experiment ($n = 6$) with purified B-CLL cells are shown in left panel. A gate based on forward and side scatters was used to evaluate monocytes Ca^{2+} mobilization in PBMC from the same patient (right panel). (C) Protein tyrosine phosphorylation induced by FcγRIIA crosslinking was evaluated by Western blot ($n = 6$) in B-CLL cells and PBMC from the same patient (right panel). Cells (2×10^6) were cultured with or without IV.3 Fab, resuspended in complete medium containing GAM and cultured at 37 °C. Reaction was stopped at the times indicated by adding cold PBS. Western blot was performed as it was described in Section 2. Tyrosine phosporylated proteins were detected by direct immunoblotting using the antibody 4G.10 and HRP-conjugated anti-mouse IgG. The molecular mass of protein standards is shown. The same membrane was blotted with MoAb anti-actin to compare the total amount of protein in each sample.

3.2. *Functional analysis of FcγRIIB expressed on B-CLL cells*

The best known function of FcγRIIB is its inhibitory capacity, which has been commonly studied in the context of BCR signaling. Thus, FcγRIIB is able to diminish activation

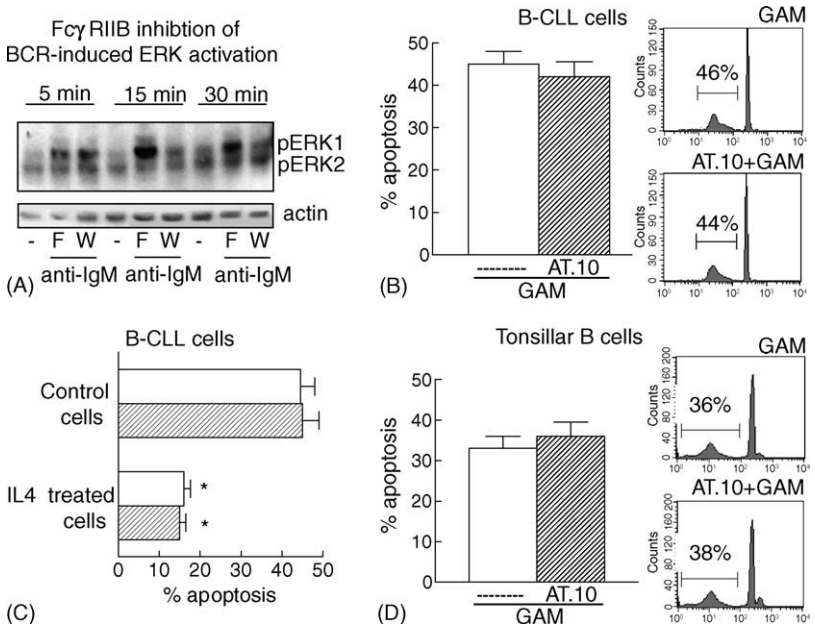

Fig. 2. Functional analysis of FcγRIIB. (A) FcγRIIB inhibition of BCR-induced ERK1/2 phosphorylation was evaluated by Western blot ($n = 6$). B-CLL cells (2×10^6) were cultured at 37 °C with 15 μg/ml of anti-IgM Fab′$_2$ (F) or whole molecule (W). Antibodies were carefully titered to ensure that equivalent molar concentration of intact IgG and Fab′$_2$ were used. Western blot was performed as it was described in Section 2 and antibodies against phospho-ERK1/2 were used in the immunoblotting. The same membrane was blotted with MoAb anti-actin to compare the total amount of protein in each sample. (B) Apoptosis induction upon FcγRIIB homoaggregation was evaluated in purified B-CLL cells negative for FcγRIIA. Cells were cultured with (hatched bar) or without (open bar) AT.10 for 15 min at room temperature, washed and then cultured in complete medium containing goat anti-mouse IgG Fab′$_2$ (GAM). Results obtained at 96 h of culture by fluorescence microscopy are expressed as mean ± S.E.M., $n = 15$. Apoptosis quantitation by flow cytometry shows the percentage of nuclei with hypodiploid DNA content. Histograms of a representative experiment are shown. (C) B-CLL cells treated with IL4 (50 ng/ml) were cultured with (hatched bars) or without (open bars) AT.10 and GAM, as described above. Apoptosis was daily evaluated by fluorescent microscopy. Results obtained at 96 h of culture are expressed as mean ± S.E.M., $n = 12$, (*) statistical significance ($p < 0.01$) compared to control cells. (D) Apoptosis induction upon FcγRIIB homoaggregation was evaluated in purified tonsillar B cells as described above. Results obtained at 48 h of culture by fluorescence microscopy are expressed as the mean ± S.E.M., $n = 4$. A representative experiment of apoptosis quantitation by flow cytometry is also shown.

signals initiated by BCR crosslinking such as tyrosine phosphorylation of a broad range of proteins, including ERK1/2 [4,5]. In order to analyze whether FcγRIIB is functional in B-CLL cells, we evaluated its ability to inhibit BCR-induced ERK1/2 activation. These experiments were carried out with B-CLL samples that readily respond to BCR aggregation, since, as previously reported [15–17], we found that a significant proportion of B-CLL clones were anergic to stimulation via BCR. ERK1/2 phosphorylation was analyzed by Western blot in purified leukemic cells treated with anti-IgM Fab′$_2$ to specifically cluster BCR, or anti-IgM whole molecule to co-cluster BCR and FcγRIIB. As shown in Fig. 2A, co-clustering of BCR and FcγRII with anti-IgM whole molecule resulted in a significant inhibition of ERK1 phosphorylation, indicating that B-CLL FcγRIIB is able to transduce inhibitory signals. Although the anti-IgM antibodies were carefully titered to ensure that equivalent molar concentration of intact IgG and Fab′$_2$ were used, we performed further experiments to rule out that the diminished signaling found with anti-IgM whole molecule might be due to a lower activity of the antibody. To this aim, we used heat agreggated human IgG (aIgG) to block the receptor before triggering BCR with anti-IgM whole molecule. We found that anti-IgM whole molecule (15 μg/ml) was able to induce a stronger ERK phosphorylation in B-CLL previously treated with aIgG compared to untreated B-CLL cells (data not shown).

In addition to its well-known inhibitory capacity, it was previously reported that murine FcγRIIB can also deliver pro-apoptotic signals upon homoaggregation [6]. Taking into account that B-CLL cells express high levels of functional FcγRIIB, we asked whether FcγRIIB crosslinking could induce B-CLL cell apoptosis. To this aim, we induced selective ligation of FcγRIIB in B-CLL samples negative for FcγRIIA, by using the pan-FcγRII MoAb AT.10 plus GAM and daily evaluated apoptosis as described in Section 2. As shown in Fig. 2B, there was no significant differences in the apoptotic rates of control and treated cells. Comparable results were obtained when platebound AT.10, aIgG or platebound human IgG were used to extensively crosslink FcγRIIB (not shown). These findings indicate that FcγRIIB homoaggregation was unable to trigger B-CLL apoptosis. In an attempt to overcome this lack of response, we used IL-4, a cytokine that was shown to favour apoptosis induced by FcγRIIB crosslinking in murine B lymphocytes [6]. To this aim, purified B-CLL cells were incubated in the presence of IL-4 for 24 h and then specific homoaggregation of FcγRIIB was induced as described above. In agreement with previous reports [28–30],

we found that IL-4 markedly inhibited spontaneous B-CLL cell apoptosis (Fig. 2C). More importantly, we observed that this protective effect was not affected by FcγRIIB homoaggregation.

Given the well-known resistance of B-CLL cells to apoptosis [9–11], the inability of FcγRIIB to deliver pro-apoptotic signals might be a particular feature of the leukemic cells. To our knowledge, whether human FcγRIIB is able to induce pro-apoptotic signals was not evaluated yet. Therefore, we carried out experiments using non-malignant B lymphocytes from human tonsils and observed that homoaggregation of FcγRIIB was completely unable to promote B cell apoptosis (Fig. 2D). These results suggest that the inability of FcγRIIB to induce B-CLL apoptosis is not a particular characteristic of leukemic cells but rather it seems that murine and human FcγRIIB trigger different signaling pathways upon homoaggregation.

4. Discussion

FcγRII isoforms are cell surface receptors for antigen–antibody complexes which display coordinate and opposite roles in immune responses depending on their cytoplasmic regions [1–3]. As we have previously reported [13], we find that leukemic B cells from a proportion of B-CLL patients aberrantly express the myeloid FcγRIIA. We here demostrate that specific crosslinking of FcγRIIA on B-CLL cells did not result in activation signals as occurs in myeloid cells. Thus, neither Ca^{2+} mobilization nor protein tyrosine phosphorylation were observed in purified leukemic lymphocytes upon FcγRIIA homoaggregation. Different factors could account for FcγRIIA nonresponsiveness in B-CLL cells. The simplest explanation might be the expression of a defective FcγRIIA protein. However, an impaired recruitment of the receptor to membrane rafts can also explain our findings. Indeed, the association of FcγRs to membrane rafts represents the initial step for receptor signaling, and is in these rafts where FcγRIIA coexists with Src family kinases and undergoes tyrosine phosphorylation [31]. Whether FcγRIIA is normally recruited to membrane rafts in B-CLL cells remains to be determined. Finally, the possibility exists that FcγRIIA signaling is inhibited in B-CLL cells. It has been reported that, upon FcγRIIA crosslinking, signaling cascade may be suppressed by the recruitment of phosphatases, such as SHIP or SHP [32]. Our own preliminary results suggest that SHIP and SHP expression is higher in B-CLL cells compared to normal B cells. Experiments are in progress in order to explore whether the over-expression of these phosphatases may account for FcγRIIA unresponsiveness.

In regard to the aberrant expression of FcγRIIA in B-CLL cells, it should be mentioned that other myelomonocytic antigens, such as CD14, CD13, CD11c or CD11b, have been associated with more advance disease and shorter overall survival [33–35]. In our study, seven out of 16 patients with indolent disease (RAI O-I) and three out of five patients with intermediate disease (RAI II) expressed FcγRIIA, whereas five out of six patients with aggressive disease (RAI III–IV) were positive for the myeloid FcγRIIA (Table 1). Although, these data suggest that FcγRIIA could be preferentially expressed in B-CLL cells from patients with more advanced disease, this observation will need to be rigorously confirmed using larger numbers of B-CLL patient samples.

Concerning FcγRIIB, we have previously reported that B-CLL cells express comparable or even higher levels of FcγRIIB than normal B lymphocytes [12]. We here show that: (1) FcγRIIB is functional in B-CLL cells since it effectively diminished BCR-triggered ERK1 activation, and (2) FcγRIIB homoaggregation does not generate proapoptotic signals, as was described for murine B cells [6]. This lack of effect is unlikely to be due to a particular resistance of leukemic cells to apoptosis since we found that, in normal B lymphocytes, the engagement of FcγRIIB was also unable to modify cell survival. These negative findings suggest that different signaling pathways may be triggered in human and murine B cells upon FcγRIIB homoaggregation.

In normal B cells, inhibitory signaling through FcγRIIB can be triggered in vivo by antigen–antibody complexes that simultaneously bind to the BCR through free antigenic epitopes and to FcγRIIB via Fc fragment of antibody. Of note, it has been reported that upon BCR-FcγRIIB aggregation, not only BCR mediated [1,4,5,36], but also signals triggered by other receptors are subject to inhibition via FcγRIIB [37]. Because a significant proportion of B-CLL clones express polyreactive BCR [38–42] leukemic cells may interact with a variety of IgG immune complexes, which could activate FcγRIIB inhibitory signaling.

In conclusion, our results show that FcγRIIB, but not FcγRIIA is biologically active in B-CLL cells and support the possibility that recognition of immune complexes influences leukemic cell physiology in vivo.

Acknowledgements

Contributions. All the authors contributed substantially to the study. G. Arrosagaray and J. Snachez-Avalos provided B-CLL samples and contributed in the design of the work. P. Fernandez-Calotti and J. Sanjurjo contributed in the analysis and interpretation of the data. R. Gamberale created the tables and figures and wrote the article, which was critically revised by M. Giordano and J. Geffner. M. Giordano was involved in the project conception and design and gave the final aproval of the version to be published. R. Gamberale takes primary responsibility for the paper. Authors reported no conflict of interest.

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