A functional BCR in Human IgA and IgM Plasma Cells

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Running title: BCR is expressed on the surface of IgA and IgM but not IgG plasma cells
Key Point

- IgA and IgM human plasma cells express a functional BCR on their cell surface and can therefore respond to antigenic stimulation.

Abstract

Plasma cells are terminally differentiated cells of the B cell lineage that secrete antibodies at high rate and are thought to lack the expression of the BCR. Here, we report that human IgA and IgM unlike IgG plasma cells express a membrane functional BCR associated with the Igα/Igβ heterodimer. BCR crosslinking on IgA and IgM plasma cells led to Ca2+ mobilization, ERK1/2 and AKT phosphorylation and impacted survival of IgA plasma cells. These findings demonstrate a significant difference between human IgG, IgM or IgA plasma cells and suggest that the IgA plasma cell repertoire may be modulated by specific antigens with implications for the regulation of the mucosal immune system.
Introduction

B cells express clonotype-specific surface immunoglobulins (Ig) which associate to the Igα/Igβ heterodimer to constitute the B cell receptor (BCR).1,2 Binding of the specific antigen to the BCR triggers a signaling cascade which leads, in concert with other signals to cell activation, proliferation and generation of memory B cells and plasma cells (PCs).3,4 BCR expression is required for memory B cells survival 5, but is thought to be lost on mature plasma cells due to a secretory switch in the Ig mRNA.6

Long lived PCs persist in bone marrow (BM) and, in the case of IgA PCs, in mucosal tissues, homing to specific niches and continually secreting antibodies thus maintaining serological memory.7-10 Serum IgG antibodies and consequently IgG PCs can persist for a lifetime, although displacement by newly generated PCs9 and susceptibility to FcγRIIB-mediated apoptosis11 increase their turnover. In contrast, the IgA PCs present in the lamina propria (LP) show a high turnover rate.12 These findings suggest different mechanisms of regulation of IgG and IgA PCs.

Methods

Cells cultures

Human tissues were obtained according to the rules of the Cantonal Ethic committee (Comitato Etico Cantonale, CH-6501 Bellinzona). This study was conducted in accordance with the Declaration of Helsinki. Bone marrow plasma cells (BM-PCs) were isolated from patients undergoing hip surgery using CD138-PE and anti-PE magnetic beads and further purified by cell sorting when cultured for ELISPOT assay. Lamina propria plasma cells (LP-PCs) were isolated from colon tissue obtained from healthy areas of surgically resected specimens and were enriched by magnetic selection of CD38 positive cells. Circulating memory B cells were isolated by cell sorting as CD19+
CD27+. PCs were cultured in complete RPMI supplemented with 10% FCS and 10ng/ml of recombinant IL-6. For in vitro differentiation, total memory B cells were pulsed for 16h with CpG 2006 and plated 5 days on a monolayer of immortalized mesenchymal stromal cells. Survival of BM-PCs was evaluated by ELISPOT on 96 well plates coated with anti-Fc antibodies. Detection of spots was performed with biotinylated anti-Fc or light chains followed by peroxydase-labelled streptavidin and 3-amino-9-ethylcarbazole.

Antibodies

Ig where detected by F(ab)2 fragments of goat anti human heavy or light chains (all from Southern Biotech). Purified anti-phospho-ERK, anti-phospho-Syk Y525-526, anti-phospho AKT (from Cell signaling) as well as anti-Blimp-1 and anti-Pax-5 (from Santa Cruz) were revealed by goat anti-Rabbit-Alexa Fluor 488 (Invitrogen). BCR cross-linking was performed with purified F(ab)2 fragments of goat anti-κ and anti-λ chains antibodies (from Southern Biotech), allowing the homogeneous and concomitant cross-linking of all BCR.

Cytometry and microscopy

BM and in vitro differentiated PCs were detected as CD138+/icIg high. Colon LP-PCs were detected as CD3-/CD20-/CD38 high/icIgA high. Staining for transcription factors and phosphorylated molecules were performed using 4% formaldehyde fixation followed by permeabilization with 90% cold methanol. Internalization of the Ig molecules was evaluated by the disappearance of light chains staining after cross-linking with anti-Fc fragment antibodies at 4°C (for control) and 37°C. Staining intensities were expressed
as ratio of mean fluorescence intensity (rMFI) to control staining to correct for differences in background. BCR internalization was evaluated by confocal microscopy 20 min following cross-linking with anti-IgA-Cy5 at 37°C or 4°C as control. Surface IgA+, surface IgM+ and surface IgA-IgM- BM-PCs were isolated by cell sorting; Igα, Igβ, surface and secreted Ig cDNA were quantified by RT-PCR.

Results and discussion

To develop an in vitro culture method that would support PC survival, we plated CpG-activated memory B cells on monolayers of BM derived MSC. In these cultures, a fraction of activated B cells acquired CD138 and express high levels of intracellular Ig, consistent with PC differentiation (Figure 1A, upper panel). Surprisingly, while IgG PCs down-regulated surface IgG expression, IgA and IgM PCs expressed their respective isotype both intracellularly and on the plasma membrane (Figure 1A, lower panel). Importantly, concordant Ig were also detected on the plasma membrane of IgA and IgM but not IgG PCs isolated from BM or colon LP indicating that this property is a characteristic of PCs present in their physiological niches (Figure 1B and C). These results were confirmed by qPCR (supplementary figure 1) and suggest that the complete switch from surface to secreted Ig is limited to IgG PCs.

To dissect a possible heterogeneity among PCs subsets, we analyzed the expression of the BCR-associated Igα and Igβ and of master transcription factors Blimp-1 and PAX-5 that control PC and B cells differentiation state. Igα and Igβ mRNA were detected by qRT-PCR in IgA, IgM and IgG BM-PCs, albeit at reduced level when compared to memory B cells (Figure 1D). Furthermore, antibodies directed against the intracellular
portion of Igα and Igβ stained all BM-PCs irrespective of the Ig isotype (Figure 1E). In addition, all BM-PCs expressed Blimp-1 but not PAX-5, HLA-DR, CD62L or Ki-67 (Figure 1F and not shown). These results are consistent with the phenotypic characterization of the cells as terminally differentiated PCs and suggest that all PCs express Igα and Igβ, although at reduced levels.

To assess the functionality of the BCR present on IgA and IgM PCs, we measured, at the single cell level, signaling events triggered by BCR cross-linking. In IgA and IgM – but not IgG – PCs from BM or LP, BCR cross-linking led to the phosphorylation of Syk at tyrosines 525-526 present in the catalytic site, the level of phosphorylation being comparable to that observed in memory B cells (Figure 2A). Furthermore, IgA and IgM BM-PCs as well as IgA LP-PCs showed BCR-induced ERK1/2 phosphorylation with dose response and kinetics comparable to those observed in activated memory B cells (Figure 2B, C and data not shown). Only κ- or λ-expressing cells responded to anti-κ or anti-λ antibodies respectively showing that ERK phosphorylation is highly specific and occurs directly through BCR and not through Fc receptors in IgA and IgM BM-PCs (Figure 2D and not shown). Finally, an increase in intracellular [Ca^{2+}] was detected by flow cytometry in IgA and IgM BM-PCs by concomitant staining and cross-linking of the BCR (supplemental Figure 2A and B). Taken together, these results indicate that human IgA and IgM but not IgG PCs express a functional BCR.

The endocytosis of the BCR, that allows B cells to take up and present antigens on MHC class II molecules, has been shown to regulate also AKT phosphorylation, a pathway involved in control of cell survival. When compared to memory B cells, IgA PCs showed a slightly reduced capacity to internalized the BCR (more pronounced in
IgM PCs - Figure 2E), nonetheless internalized IgA could be detected as multiple spots distributed all over the cytoplasm (Figure 2F). When cross-linked with anti-light chains antibodies, IgM and in particular IgA PCs from BM or colon LP showed increased AKT phosphorylation (Figure 2G and not shown). Furthermore, upon BCR cross-linking, only IgA BM-PCs up-regulated CD44, an adhesion molecule involved in PC survival19 (Figure 2H).

To test whether BCR cross-linking might affect survival of IgA PCs, we cultured IgA and IgM BM-PCs in the presence of cross-linking antibodies and measured by ELISPOT their survival after 4 days. An increase survival of IgA PCs (but not of IgM PCs) was evident at low cross-linking antibodies doses (Figure 2I) with no sign of cell division (Figure 2J, left panel). At high doses, both IgA and IgM recovery was significantly impaired (Figure 2I) in an apparently BCR dependent manner (Figure 2J, right panel).

This study highlights an unexpected property of IgM and IgA PCs: the surface expression of a functional BCR. Importantly, this was shown for in vitro differentiated PCs as well as for PCs isolated ex-vivo from human BM and gut LP. To note, surface IgA expression has been reported in mice gut LP-PCs.20 These findings imply that IgM and IgA unlike IgG PCs can directly respond to their antigen. Moreover, BCR cross-linking can impact positively on the survival of IgA PCs. Our observation may partially be relevant to understand the dynamics of IgA PCs in the gut and their capacity to adapt to commensals.12 We propose that the selection of IgA PCs (and most likely IgA plasmablasts) in the lamina propria could be partially due to the capacity of these cells to respond positively to their antigen at low doses, a mechanism that would favor PCs whose antigen is present at the mucosal surface.
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Authorship

Contribution: D.P. designed and performed experiments and analyzed data. E.M. performed experiments and analyzed data. M.B. and G.G. analyzed data. A.L. and F.S. analyzed data and wrote the manuscript. D.J. designed and coordinated the study, performed and analyzed experiments and wrote the manuscript.

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Figure legends

Figure 1. Surface BCR expression on human IgM and IgA plasma cells. (A) In vitro differentiated memory cells were stained for CD138 and membrane (mb) and intracellular (ic) Ig; one representative experiment of 4. (B) Expression of ic and mb IgG, IgA and IgM in CD19<sup>+</sup>CD138<sup>+</sup>-gated BM-PC; one representative experiment of 3. (C) Expression of CD20 and CD138 on the CD38-enriched fraction of colon LP (left panel). Expression mb and ic IgA in CD3<sup>-</sup>/CD38<sup>high</sup> LP plasma cells (right panel); one experiment of 2. (D) Igα and Igβ mRNA in BM-PC and memory B cells normalized to 18S RNA. Mean + SEM of three donors. (E) Expression of intracellular epitopes (grey histograms) of Igα and Igβ on IgG, IgA and IgM BM-PCs. Control staining, open histograms; one experiment of 3. (F) Expression of Blimp-1 and Pax-5 on BM-PCs and memory B cells. Mean of rMFI + SEM of duplicates; one experiment of 3.

Figure 2. Human IgM and IgA plasma cells express a functional BCR. (A) Increase in phospho-Syk<sup>Y525-526</sup> staining in BM-PC (black bars), memory B cells (white bars) and LP-PC (grey bar) following cross-linking with 2.5 µg/ml anti-light chain F(ab’)<sub>2</sub> antibody fragments. Mean of rMFI + SEM of duplicates. PC gates as in Fig. 1. One experiment of 3. (B) ERK1/2 phosphorylation of BM-PC (black circles) and memory B cells (white triangles) 15 min. after stimulation with increasing doses of anti-light chain antibodies; one experiment of 3. (C) Levels of ERK 1/2 phosphorylation in LP-PC cultured for 15 min. in the presence or absence of anti-IgA F(ab’)<sub>2</sub> antibodies. Mean of rMFI + SEM of duplicates; one representative experiment of 2. (D) Levels of ERK 1/2 phosphorylation of κ (black bars) or λ (white bars) IgA BM-PCs after 15 min. in the presence of 2.5 µg/ml anti-κ or -λ light chain F(ab’)<sub>2</sub> antibodies. Mean + SEM of
duplicate; one experiment of 3. (E) Internalization of IgM and IgA on BM-PCs and memory B cells cultured with 2.5 μg/ml anti-IgM or IgA heavy chain F(ab’)2 fragments. Shown is the surface staining of light chains after 20 min at 4°C (black bars) or 37°C (white bars). Mean of fold expression to control (concomitant staining of heavy and light chains at 4°C) + SEM of duplicates; one experiment of 3. (F) Confocal images of IgA BM-PC and memory B cells incubated for 20 min. with anti-IgA-Cy5 (green) and counterstained with CD45-Alexa fluor 700 (red) as membrane marker and DAPI (blue). Images are representative of 2 independent experiments. Quantification of the ratio of internal vs membrane IgA (right panel). (G) Expression of phospho-AKT (Ser 473) on IgA and IgM BM-PCs cultured for 20 min. with 2.5 μg/ml anti-light chain F(ab’)2 antibodies. Mean of rMFI + SEM of duplicates; one representative experiment of 3. (H) Expression of CD44 on IgG, IgA and IgM BM-PCs cultured for 12 h. with increasing doses of F(ab)2 anti-light chain antibodies. Mean of rMFI + SEM of duplicates; one experiment of 3. (I) Survival of IgA and IgM BM-PCs cultured for 4 days in the presence of increasing doses of anti-light chain F(ab’)2 antibodies. Mean of % input cells + SEM of duplicates; one representative experiment of 3. (J) Cell tracer dilution of IgA BMPCs after 4 days in culture with (lower histogram) or without (upper histogram) 0.5 μg/ml of anti-light chains F(ab’)2 antibodies (left panel). One experiment out of 2. Survival of κ (black bars) or λ (white bars) IgA BM-PCs upon cultured for 4 days in the presence of 10 μg/ml anti-κ or -λ light chain F(ab’)2 antibodies. Mean + SEM of duplicate; one experiment of 3 (right panel). * p<0.05. ** p=0.01 in Student’s T test.
Pinto et al. Figure 2

A

- BM-PC
- Memory
- LP-PC

Fold increase to control

IgG IgA IgM IgA

B

- Memory B
- BM-PC

P-ERK 1/2 (mMFI)

anti-κ + anti-λ (µg/ml)

0 0.1 1 10 0.1 1 10 0.1 1 10

IgG IgA IgM

C

LP-PC

P-ERK (mMFI)

anti-IgA - +

D

p-ERK (MFI)

10 1.0 1.5 2.0

anti-κ anti-λ

E

Fold expression to control

IgA IgM

F

4°C 37°C

4°C 37°C

Memory BMPC Memory BMPC

Ratio Int. Mb (A.U.)

G

phospho-AKT (MFI)

1.0 2.0 3.0

IgA IgM

H

CD44 (MFI)

100 200 300

0 10^{-1} 10 10^{1} 10^{2} 10^{3} 10^{4} 10^{5}

anti-κ + anti-λ (µg/ml)

IgG IgA IgM

I

ASC (% input)

40 60 80

0 10^{-1} 10 10^{1} 10^{2} 10^{3}

anti-κ + anti-λ (µg/ml)

J

Control

Anti-κ+λ

0.5 µg/ml

Cell tracer

IgA ASC (% control)

0 20 40 60 80 100

anti-κ anti-λ

* p < 0.05

** p < 0.01

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