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Dual isotype expressing B cells [κ(+)/λ(+)] arise during the ontogeny of B cells in the bone marrow of normal nontransgenic mice

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Abstract

Central to the clonal selection theory is the tenet that a single B cell expresses a single receptor with a single specificity. Previously, based on our work in anti-phosphocholine transgenic mouse models, we suggested that B cells escaped clonal deletion by coexpression of more than one receptor on their cell surface. We argued that "receptor dilution" was necessary when: (i) the expressed immunoglobulin receptor is essential for immune protection against pathogens and (ii) this protective receptor is autoreactive and would be clonally deleted, leaving a hole in the B cell repertoire. Here, we demonstrate that dual isotype expressing B cells arise during the normal ontogeny of B cells in the bone marrow and populate both the spleen and peritoneal cavity of nontransgenic mice. Furthermore, single cell analysis of the expressed immunoglobulin light chains suggests that receptor editing may play a role in the generation of a significant fraction of dual isotype expressing B cells.
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1. Introduction

The immune system maintains a delicate balance between the necessity to generate a naive repertoire with sufficient diversity to recognize a wide range of nonself or foreign antigens while minimizing the repertoire of receptors that are autoreactive and pose a potential threat to the host. One model, "the clonal selection theory," proposed by Burnet [1] currently serves as the basis for considering this problem. Central to this model is the postulate that a single cell expresses a single receptor with specificity for a single antigen which was supported by the early studies of Pernis et al. [2] and Cebra et al. [3]. These studies demonstrated that virtually all B cells expressed a single heavy chain allele, a phenomenon referred to as allelic exclusion.

It is easy to see how the single cell, single receptor, single specificity model provides the basis for understanding both positive and negative clonal selection. The importance of negative selection or clonal deletion in screening and eliminating autoreactive B cells has been experimentally established in numerous transgenic mouse models [4–10]. However, a number of other mechanisms for screening and controlling autoreactive B cells have been described including: (1) the induction of a state of functional anergy and segregation and isolation of the autoreactive B cells to immunologically privileged sites such as the peritoneal cavity [11–14], and (2) receptor editing and/or receptor revision, a process that rescues the cell by replacing the offending autoreactive immunoglobulin light chain or heavy chain [15–19]. As a result of receptor editing or revision, the original autoreactive specificity is lost.

Exceptions to this single cell, single receptor, single specificity have been observed. Studies examining human lymphoid neoplasias, murine lymphomas, and experimentally induced plasmacytomas in mice have described single

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B cells expressing more than one immunoglobulin light chain on their surface [20–25]. Hybridomas that express more than one light chain (with varying degrees of stability) have been observed [26–28] and dual light chain expressing B cells have been reported in numerous conventional and targeted transgenic mouse models including; mice generated by nuclear transfer [29], the human and mouse kappa chain polymorphic constant regions mouse model [30], the anti-DNA mouse model [15,31–34], the anti-MHC mouse model [17,35], and the anti-phosphocholine mouse model [36]. In addition, the rare dual light chain expressing B cell has been reported in normal human B cell populations [37–41] as well as normal mouse B cell populations [28,42].

Very little is known about the ontogeny and population characteristics of these dual light chain expressing cells. In our previous work with the anti-phosphocholine (PC) transgenic mouse model, we proposed that dual light chain expressing B cells were a part of the normal B cell population that used coexpression to escape clonal deletion. We suggested that coexpression altered the number of autoreactive receptors expressed on the B cell and thereby raised the signaling threshold that normally resulted in tolerance induction. We referred to this mechanism as "receptor dilution" and suggested that it was a generalized mechanism for rescuing B cells that expressed a receptor that was autoreactive but essential for immune protection [36]. We also showed that coexpression not only rescued the autoreactive PC-binding B cells but also reversed the functional anergy of the PC-specific B cells and allowed the rescued cells to develop into conventional CD23(+) B2 B cells that could then expand and populate the spleen. Furthermore, these dual receptor expressing cells participated in protecting the mouse against *Streptococcus pneumoniae*. In the current study, we have identified a small but real population of dual isotype expressing B cells in the bone marrow, spleen, and peritoneal cavity of normal, unmanipulated, nontransgenic mice. Examination of expressed immunoglobulin heavy chains and light chains from single dual isotype expressing cells provides evidence that dual isotype expressing B cells arise during the normal ontogeny of bone marrow-derived B cells and that receptor editing may play a role in their generation. Immunophenotype analysis suggests that the dual isotype expressing B cells are not preferentially differentiated into any individual B cell population (e.g., marginal zone cells, B1 B cells or conventional follicular B cells) but rather the dual isotype expressing B cell populations consist of similar proportions of each of these populations as those observed in κ(+) only B cells.

2. Materials and methods

2.1. Mice, preparation of cells, and antibodies

Single cell suspensions of bone marrow, peritoneal, and splenic lymphocytes were prepared from 2- to 6-month-old C57BL/6 mice as described previously [43,44]. All studies have been reviewed and approved by the Gerontology Research Center Animal Care and Use Committee. PE conjugates of anti-B220 (clone RA3-6B2), anti-κ (clone HB58), anti-CD23 (clone B3B4), anti-δ (clone 11–26), and anti-IgM (clone 331.12) were prepared by Molecular Probes (Eugene, OR). Anti-IgM was conjugated to FITC as previously described [43] and anti-κ and anti-B220 Cy5 conjugates were prepared according to the manufacturer's instructions (Biological Detection Systems, Pittsburg, PA). Anti-CD11b-PE (clone M1/70), anti-λ-FITC (clone R26-46), and anti-CD5-PE (clone 53-7.3) were purchased from Pharmingen (San Diego, CA).

2.2. Immunophenotype analysis

Single cell suspensions were immunophenotyped as previously described [43,44]. Four color, six parameter list-mode data (20,000 events) were acquired using a FACSCalibur, (BD Immunocytometry Systems, San Jose, CA) and the resulting data analyzed using the WinList Ver.4 software package from Verity Software House (Topsham, ME).

2.3. Magnetic bead selection of λ(+) cells and single cell sorting of κ/λ double positive cells

Single cell preparations of splenic lymphocytes were stained as described above with anti-λ-FITC. The stained cells were washed, suspended to 10^7 cells/90 μl, and passed through 40-μm nylon screen. Anti-FITC magnetic beads (Miltenyi Biotec, Auburn, CA) were added to a final concentration of 10 μl of beads/10^7 cells. λ(+) cells were selected on a RS+ column (Miltenyi Biotec) as recommended in the accompanying protocol, stained with anti-κ-PE, and bulk sorted twice on a FACStar^{plus} equipped with an automatic cell deposition unit (BD Immunocytometry Systems) to enrich for κ(+)/λ(+) cells. Bulk sorted cells were analyzed using a FACScan (BD Immunocytometry Systems) and dual isotype positive single cells were directly sorted into 96-well plates containing 10 μl of 0.5× PBS/10 mM DTT and 10 U of RNasin (Promega, Madison, WI) per well. The plates were briefly spun and the cells were stored at −70 °C until used.

2.4. RT and nested PCR amplification of κ(+)/λ(+) single cells

Initial RT-PCR amplification of single cells for either V_H or V_κ genes was performed using the SuperScript One-Step RT-PCR kit from Gibco-BRL (Life Technologies, Gaithersburg, MD). Primers for amplification of the V_H genes were MSVHE and MSCmuE and for the V_κ genes were MSVKM and MSCK1 [45]. Primer concentrations were 0.4 μl of 25 pmol/μl in a final RT-PCR volume of 50 μl. cDNA synthesis and PCR amplification were performed using a TouchDown thermocycler (Thermo Hybaid, Middlesex, UK) equipped with a 96-well block and heated lid. cDNA synthesis was performed by incubation of the reaction at 50 °C for 15 min followed by an incubation for