Available online at www.sciencedirect.com
ScienceDirect
Immunology Letters 117 (2008) 96–105
Immunology Letters
www.elsevier.com/locate/

Analysis of B cell selection in the germinal center reaction during a T-dependent antibody response at a single cell level

Takahiro Okazawa, Masaki Magari, Takafumi Kimoto, Emi Kouyama, Hitoshi Ohmori, Naoki Kanayama *

Department of Bioscience and Biotechnology, Graduate School of Natural Science and Technology, Okayama University, 3-1-1 Tsushima-Naka, Okayama 700-8530, Japan

Received 10 November 2007; received in revised form 23 December 2007; accepted 6 January 2008
Available online 4 February 2008

Abstract

The quasimonoclonal mouse is useful to examine B cell selection during T-dependent antibody (Ab) responses because of its limited B cell populations mainly expressing the knockin 17.2.25 V_H-encoded H chain (V_HT) paired with the λ1 or λ2 L chain. It has been reported that both two V_HT/λ1 and V_HT/λ2 B cell populations responded to a T-dependent antigen conjugated with a hapten *p*-nitrophenylacetyl (*p*NP), but only V_HT/λ2 B cells differentiated to secrete high affinity anti-*p*NP IgG Abs by acquiring a critical mutation (T313A) in the V_HT. The V_HT/λ2 B cells may be more potent in migrating to the germinal centers (GCs) due to about 50-fold higher affinity for *p*NP than V_HT/λ1 B cells. Here, to uncover how V_HT/λ2 B cells were preferentially recruited for affinity maturation during the anti-*p*NP Ab response, we examined the L chain usage and mutation frequency of V_HT$^+$ GC B cells at a single cell level. V_HT/λ2 B cells bearing the unmutated V_HT gene were found in the GCs more frequently than V_HT/λ1 and mutated V_HT/λ2 counterparts in an early phase of the Ab response. In the course of the GC reaction, the number of V_HT/λ2 B cells that mutated their V_HT genes preferentially expanded, and finally V_HT/λ2 B cells bearing the T313A mutation occupied V_HT$^+$ GC B cell population. Thus, it is suggested that B cells with a higher affinity were selected not only for entry to the GCs but also in the affinity maturation process during a T-dependent Ab response.

Keywords: Affinity maturation; B cell antigen receptors; B lymphocytes; Germinal centers

1. Introduction

During immune responses against T-dependent antigens (Ag) in higher vertebrates, the affinity of antibodies (Ab) for Ags is improved accompanying class switch recombination (CSR) from IgM to others including IgG. The process is known as affinity maturation, which is strongly dependent on the germinal centers (GC) that are transiently formed from Ag-stimulated B and T cells in the follicular region of secondary lymphoid tissues [1–5]. GC B cells undergo somatic hypermutation (SHM) of V region genes coupled with the positive selection of mutant clones whose B cell Ag receptors (BCR) acquire higher affinity [6,7]. Activation-induced cytidine deaminsase (AID) is essential for both CSR and SHM [8,9]. In the GC reaction, competition between GC B cells for capturing Ags is thought to occur and B cells with improved affinity are considered to survive in collaboration with CD4$^+$ T helper and follicular dendritic cells, thereby leading to the retention of B cells secreting higher affinity Abs [1–5,10].

The affinity of BCR may have a role in competition between antigen-stimulated B cells for being selected for the GCs that affinity maturation is undergoing [11–13]. Studies using immunoglobulin (Ig) transgenic B cells specific to hen egg lysozyme (HEL) or a hapten (4-hydroxy-3-nitrophenyl)acetyl (NP) revealed involvement of the BCR in the early decision of T-dependent B cell responses. In the analysis of competition between high- and low-affinity NP-specific B cells for entering GCs, it has been suggested that the higher affinity of the BCR leads to the Ag-driven stringent selection and clonal expansion of the B cells in the GCs [14]. However, in the experimental model using anti-HEL B cells, high affinity for Ag was not strictly required for GC entry of Ag-stimulated B cells, while

* Corresponding author. Tel.: +81 86 251 8198; fax: +81 86 251 8198.
E-mail address: nkanayam@cc.okayama-u.ac.jp (N. Kanayama).

0165-2478/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.imlet.2008.01.002

that was necessary for differentiation of GC B cells to plasma cells [15,16]. B cell clones with a wide variety of affinities were also found in the GCs by day 4 during a T-dependent response for NP in wild type mice [17]. However, it remains to be elucidated how a higher affinity clone is selected in competition between antigen-specific B cells during the GC reaction.

We have previously reported that the quasimonoclonal (QM) mouse, whose B cell repertoire is restricted to two major B cell populations, is useful to analyze the clonal selection as competition of two B cell clones during a T-dependent immune response [18]. Approximately 80% of QM B cells express the NP-specific BCR that comprises the knockin V_H 17.2.25 (V_HT)-encoded H chain, which is mainly associated with the λ1 ($V_HT/λ1$) or λ2 L chain ($V_HT/λ2$) due to disruption of both κ loci, and the BCR shows about 20-fold lower affinity for an NP analog, *p*-nitrophenylacetyl (*p*NP), than for NP [18–20]. During a T-dependent anti-*p*NP Ab response, both $V_HT/λ1$ and $V_HT/λ2$ B cells could be activated and differentiate into $CD138^+$ plasma cells that secreted low-affinity anti-*p*NP IgM Abs in the early stage, but only $V_HT/λ2$ B cells underwent affinity maturation to produce high affinity anti-*p*NP IgG Abs in the later. In addition, a conserved mutation in the complementarity-determining region (CDR) 3 in the V_HT gene has been found to be critical for acquiring high affinity and could be a marker for affinity maturation [18]. Adoptive transfer of QM B cells into CGG-primed wild type mice revealed that $V_HT/λ2$ B cells more preferentially entered the GCs than $V_HT/λ1$ B cells, suggesting that B cells that received a stronger BCR signal might proceed in affinity maturation because the $V_HT/λ2$ BCR showed 50- to 100-fold higher affinity for *p*NP than the $V_HT/λ1$ counterpart [21]. Here, to make clear how the $V_HT/λ2$ B cells with a higher affinity for *p*NP were committed to affinity maturation in QM mice, we investigated selection processes of B cells in the GCs during the anti-*p*NP Ab response in detail. We developed a set of single cell PCR analyses to examine changing in the number, L chain usage, and mutation frequency of V_HT^+ GC B cells in the process of the GC reaction. It was of interest to estimate contributions of competition between $V_HT/λ1$ and $V_HT/λ2$ B cells for entry to the GCs and competition in the affinity maturation process during the Ab response in QM mice.

2. Materials and methods

2.1. Haptens and Ags

NP and *p*NP were purchased as the free acids from Tokyo Kasei (Tokyo, Japan). NP or *p*NP was conjugated to chicken γ-globulin (CGG) or bovine serum albumin (BSA) (Sigma–Aldrich, St. Louis, MO) by reacting the *N*-hydroxysuccinimide ester of each acid, as described previously [22]. Usually, CGG was conjugated with ∼25 molecules of each hapten.

2.2. Mice and immunization

QM mice (V_HT/J_H^-, $Jκ^-/Jκ^-$, $λ^+/λ^+$) were kindly provided by Dr. M. Cascalho (Department of Immunology, Mayo Medical School, Rochester, MN) [19] and used at 8–12 weeks of age. QM mice were immunized in the hind footpad with 20 μg of pNP_{25}-CGG emulsified in CFA, and bled on indicated days after immunization. All mice were treated in accordance with the guidelines approved by the Committee of Laboratory Animal Care, Okayama University.

2.3. ELISA

To assess the titer of anti-*p*NP IgG Abs in sera, ELISA was performed using 96-well microplates (Greiner, Wemmel, Belgium) coated with pNP_{20}-BSA (a high hapten density). Sera diluted 500 times were applied to the coated plates and anti-*p*NP IgG Abs bound on the plates were detected with peroxidase-conjugated goat anti-mouse IgG Ab (Zymed, South San Francisco, CA). Relative affinity of anti-*p*NP IgG Abs in serum samples was estimated by differential binding of the Abs to plates coated with 10 μg/ml pNP_5-BSA (a low hapten density) or pNP_{20}-BSA (a high hapten density) and the values were expressed as the ratio of Ab-bindings to pNP_5 over pNP_{20}, as reported previously [18,22].

2.4. BIACORE analysis

To determine the K_D values of unmutated and mutated $V_HT/λ2$ mAbs, mAbs produced by hybridoma clones G1-5 and G166, respectively, were purified from ascites with HiTrap Protein G HP (GE healthcare Amersham Biosciences, Uppsala, Sweden). The K_D values of the G1-5 and G166 mAbs were determined by surface plasmon resonance with BIACORE X (GE healthcare Biacore, Uppsala, Sweden). Approximately 2000 response units (RU) of pNP_{20}-BSA were immobilized on CM5 sensor chip. Abs at concentrations between 84 and 670 nM in HBS-EP buffer were applied over the *p*NP-BSA immobilized sensor chip. All experiments were conducted at 25 °C. Data were analyzed by BIAevaluation software (GE healthcare Biacore).

2.5. Flow cytometric analysis

Single cell suspensions were prepared from the popliteal lymph nodes of QM mice immunized with *p*NP-CGG on indicated days. Cells were stained with anti-mouse Abs in phosphate buffered saline (PBS) containing 0.2% BSA and 0.1% sodium azide. Abs used were as follows: CyChrome anti-B220 (RA3-6B2), PE anti-CD95 (Fas), FITC anti-GL7 were purchased from BD PharMingen (San Diego, CA). Stained cells were analyzed by using FACSCalibur with CellQuest software or FACSAria with FACSDiva software (BD Biosciences, Mountain View, CA).

2.6. Quantitative real-time RT-PCR analysis of AID expression

$B220^+$ B cells, $B220^+$ $GL7^-$ Fas^- B cells and $B220^+$ $GL7^+$ Fas^+ B cells were isolated from the popliteal lymph nodes by using FACSAria (BD Biosciences). Total RNA was extracted from the cells with TRIzol reagent (Invitrogen Life