Human Immunology 75 (2014) 923–929

Contents lists available at ScienceDirect

journal homepage: www.elsevier.com/locate/humimm

Rapid Communication

Retinoic acid acts as a selective human IgA switch factor

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ARTICLE INFO

Article history:
Received 23 October 2013
Accepted 23 June 2014
Available online 30 June 2014

Keywords:
Retinoic acid
IgA
Class switch
RARα
B cell

ABSTRACT

Retinoic acid (RA) is known to have several functions that lead to a potent mucosal IgA response. Nevertheless, its exact role in human IgA synthesis has yet to be elucidated. Thus, we investigated the role of RA in promoting IgA isotype switching in human B cells. We found that RA increased IgA production and the expression of germ-line IgA1 and IgA2 transcripts (GLTα1 and GLTα2). This induction occurred alongside an increase in the frequency of IgA1-secreting B cell clones, as assessed by limiting dilution analysis. Under the same conditions, RA did not increase IgM and IgG production. Am80, an agonist of RA receptor α (RARα), increased IgA production. In addition, RA activity was abrogated by LE540, an antagonist of RAR, suggesting that the RAR pathway is involved in RA-induced IgA production. Taken together, these results indicate that RA induces IgA isotype switching mainly through RARα in human B cells.

1. Introduction

Ig class switch recombination (CSR) allows a recombined variable region gene segment (VDJ) to be expressed with a new downstream heavy-chain constant region (C_H) gene. Class switching is affected by deletional recombination between switch (S) region sequences located upstream of each C_H gene except C_δ. It is generally accepted that CSR requires germ-line (GL) transcription through target S regions and expression of activation-induced cytidine deaminase (AID), an essential enzyme for Ig CSR [1,2]. CSR is directed to a particular C_H gene by cytokines that induce transcription from GL C_H genes before switch recombination of the same C_H gene [1,3].

IgA serves as a first line of defense in mucosa by inhibiting adhesion of microorganisms, removing immune complexes, and neutralizing intracellular viruses [4]. Human IgA comprises two subclasses, IgA1 and IgA2. TGF-β1 is a well-known IgA class switching factor in mouse and human [5,6]. In mouse B cells, TGF-β1 induces both germ line transcript α (GLTα)/GLTγ2b expression and subsequent CSR to IgA/IgG2b [7,8]. Similarly, in human B cells, TGF-β1 directs GLTα1/GLTα2 expression and subsequent IgA1/IgA2 secretion [9]. CD40L ligation also induces IgA CSR in the presence of IL-4 and IL-10 [9,10]. In addition, it is known that APRIL is involved in human IgA CSR [11].

Retinoic acid (RA), a vitamin A metabolite, plays an important role in the regulation of mucosal immunity [12]. Related to the IgA response, vitamin A deficiency is associated with impaired mucosal immunity due to selective loss of IgA-producing cells [13]. Studies have shown that RA enhances IgA production by LPS-stimulated mouse B cells [14] and that this activity of RA is dependent on IL-5 [15]. Similarly, RA derived from GALT-dendritic cells (DC) does not promote IgA secretion by itself, but in the presence of IL-6 or IL-5, does induce IgA expression in B cells [13]. However, we have recently shown that RA alone can specifically promote IgA isotype switching in murine B cells [16,17]. As for human Ab regulation, studies have shown that RA increases IgG production [18] but inhibits CD40L/IL-4-mediated IgE production [19]. Nonetheless, it remains unknown whether RA can affect IgA production in human B cells.

Abbreviations: CSR, class switch recombination; DC, dendritic cells; GL, germ-line; GLT, germ-line transcript; mIgA$^-$, membrane IgA negative; RA, retinoic acid; RAR, retinoic acid receptor.

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http://dx.doi.org/10.1016/j.humimm.2014.06.021
0198-8859/

In this study, we investigated the regulatory role of RA in IgA CSR in human tonsillar B cells. RA alone induced IgA production exclusively. Furthermore, at the clonal level, limiting dilution analysis was used to demonstrate the role of RA as an IgA isotype switch factor. Further, we found that the RARα mediated RA-induced IgA expression.

2. Materials and methods

2.1. Abs and other reagents

All protocols were approved by the ethics committee of Kangwon National University. Anti-IgM (DA4.4, IgG1) and anti-CD40 (G28.5, IgG1) Abs were obtained from the American Type Culture Collection (ATCC, Manassas, VA). Recombinant IL-4 was prepared in our laboratory [20]. Anti-human IgA-unlabeled

2.2. B cell preparation and cell culture

Human tonsillar B cells were prepared as described previously [21]. Mononuclear cells were subjected to two rounds of T cell depletion by rosetting with SRBCs. This resulted in $CD20^+$ B cells comprising more than 95% of the residual population, as assessed by FACSCalibur (BD Biosciences, San Jose, CA). A total of 2×10^5 -cells/well were cultured in flat-bottomed, 96-well tissue culture plates (SPL, Seoul, Korea) in 200 μl of complete medium containing anti-IgM (1 μg/ml), anti-CD40 Ab (1 μg/ml), and IL-4 (50 U/ml). When necessary, RA (Sigma–Aldrich, St. Louis, MO), TGF-β1 (R&D Systems, Minneapolis, MN), LE540 (Wako Pure Chemical Industries Ltd, Osaka, Japan), and AM80 (Tocris Bioscience, Bristol, UK) were added to cultures.

2.3. Isotype-specific ELISA and ELISPOT assay

ELISAs were performed as described previously [22]. The reaction products were measured at an absorbance of 405 nm with an ELISA reader (VERSAMAX reader, Molecular Devices, Sunnyvale, CA). Isotype-specific ELISPOT assays were performed as described previously [23]. Data are presented as the number of spot-forming cells per 2×10^5 cultured cells.

2.4. Limiting dilution analysis

For limiting dilution analysis, B cells were cultured at various cell densities in round- bottomed, 96-well plates in a final volume of 200 μl of complete medium. A total of 96 replicate cultures were set up at each cell density. Cultured wells were individually assayed for the number of IgA-secreting cells by ELISPOT assay after 6 days of culture. Cultures with spot-forming cells were scored as positive.

Calculations to determine the frequency of B cells secreting IgA were based on Poisson distribution analysis [24]. The frequency of negative responses at each input number of B cells was used to construct a plot of the $\log_{10}$ of the percentage of negative cultures verses the total number of cultured B cells. Poisson distribution laws predict a straight line that extrapolates to 100% negative responses in cultures with no IgA-secreting B cells. The frequency of IgA-secreting B cells was derived graphically, as demonstrated in the figure, by interpolating the cell number at which 37% of cultures were negative. This yields a cell number at which, on average, there is one IgA-secreting clone per culture.

The average size of IgA-secreting clones was calculated using the input number of B cells at which 37% of the cultures were negative. At this input number, the positive cultures contained an average of one precursor per culture. Clone sizes are expressed as

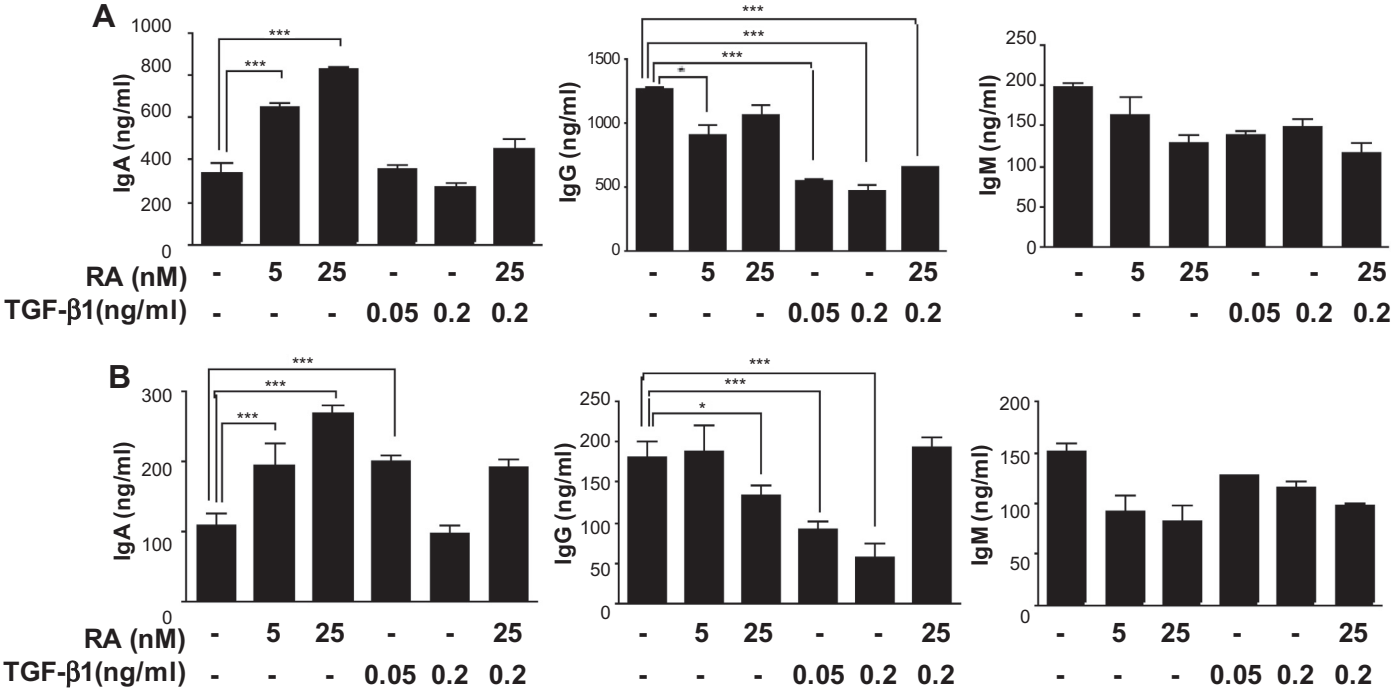

Fig. 1. Effect of RA along with TGF-β1 on Ig secretion by human tonsillar B cells. (A) Tonsillar B cells (1×10^6 cells/ml) were stimulated with anti-IgM Ab (1 μg/ml), anti-CD40 Ab (1 μg/ml), and IL-4 (50 U/ml) in the presence of RA (5 or 25 nM) or TGF-β1 (0.05 or 0.2 ng/ml). After 9 days of culture, supernatants were collected and Ig production was determined by isotype-specific ELISA. (B) Culture conditions were the same as in panel A. After 2 days of culture, cells were washed with incomplete media and fresh culture medium without RA or TGFβ1 was added. After an additional seven days of culture, supernatants were collected, and Ig production was determined by isotype-specific ELISA. Data represent the mean ± SEM of triplicate samples. ***$p < 0.001$.