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Epithelial cells are a source of natural IgM that contribute to innate immune responses

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ABSTRACT

Currently, natural IgM antibodies are considered to be the constitutively secreted products of B-1 cells in mice and humans. In this study, we found that mouse epithelial cells, including liver epithelial cells and small intestinal epithelial cells (IECs), could express IgM that also showed natural antibody activity. Moreover, similar to the B-1 cell-derived natural IgM that can be upregulated by TLR9 agonists (mimicking bacterial infection), the expression of epithelial cell-derived natural IgM could also be significantly increased by TLR9 signaling. More importantly, the epithelial cell-derived IgM was polyreactive, and it could recognize single-stranded DNA (ssDNA), double-stranded DNA (dsDNA), lipopolysaccharide (LPS), and insulin with low affinity; additionally, TLR9 agonists could enhance it in a MyD88-dependent manner. Furthermore, epithelial cell-derived IgM could bind various bacteria; therefore, it could be involved in anti-infection responses. Together, these results highlight the fact that epithelial cells are an important source of natural IgM, in addition to that produced by B-1 cells, and IgM contributes to the innate immune responses in local tissues, further demonstrating that the epithelium is a first line of defense in the protection against invading microbes.

1. Introduction

Immunoglobulin (Ig) was previously thought to be produced by only B-lineage cells. However, in the last decades, the results from a series of studies have indicated that non-B cells can also produce Igs. As early as 20 years ago, studies by our group showed that many types of non-B cancer cells, especially epithelial cancer cells, could also express Igs, including IgG, IgA and IgM (Qiu et al., 2003; Deng et al., 2006; Zhu et al., 2008; Huang et al., 2008, 2009, 2014; Zheng et al., 2009; Zhang et al., 2010; Jiang et al., 2015). Moreover, the epithelial cancer cells that expressed IgG showed growth factor-like activity that could promote cancer progression (Deng et al., 2006; Zheng et al., 2007a). Subsequently, other researchers have also demonstrated these unusual findings (Kimoto, 1998; Li et al., 2001, 2004; Babbage et al., 2006; Chen et al., 2007; Liu et al., 2007; Zheng et al., 2007b; Lee et al., 2008; Lee, 2009). Recently, growing evidence has been found to support the finding that normal non-B cells, including epithelial cells, endothelial cells, neurons, germ cells, and even monocytes, can also express Igs, such as IgG, IgA and IgM (Huang et al., 2008, 2009; Li et al., 2004; Qiu et al., 2013; Zhao et al., 2011). All of these studies indicated that the classical concept suggesting that B cells are the only source of Igs should be updated. However, the detailed physiological significance of non-B cell-derived Igs remains unclear.

Natural antibodies are preformed antibodies that are present, even in naive germ-free mice, in the absence of any exogenous antigenic exposure. Consistent with their specificities for microbial antigens, natural antibodies play an important non-redundant role in the first line of defence against bacterial and viral infections. Additionally, natural antibodies have also been shown to have specificities for self-antigens and have therefore been proposed to provide important homeostatic "house-keeping" functions. Thus far, B-1 cells that express CD5 have been considered as the major source of natural antibodies, including natural IgM, IgG and IgA (Leifer et al., 2004; Janssens and Beyaert, 2002). Particularly, B-1a cells, which are enriched in the peritoneal cavity, have been considered as the only source of natural IgM. However, the B-1a cells in the peritoneal cavity have been proven to poorly contribute to serum

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IgM levels (Holodick et al., 2010; Tumang et al., 2005), suggesting that in addition to B-1 cell, there are other cell types involved in the production of natural IgM. Recently, many epithelial cell lineages, including breast, colon, and epidermal squamous cells, were found to spontaneously express Igs, including IgG, IgA and IgM. Importantly, epithelial cell-derived Igs showed natural antibody activity. For example, the IgG and IgA that are spontaneously secreted by epidermal squamous cells showed anti-bacterial activity (Jiang et al., 2015). A cervical cancer cell line (HeLa)-derived IgM displayed natural IgM activity; it could recognize single-stranded DNA (ssDNA), double-stranded DNA (dsDNA) and lipopolysaccharide (LPS) and was upregulated by a TLR9 agonist (Hu et al., 2012). By integrating these facts, we hypothesize that in addition to B-1 cells, epithelial cells are an important source of natural antibodies, and they might play an important role in innate immunity via participating in humeral immunity.

The IgM isotype represents one of the major Ig classes in the body. IgM is also the earliest type of membrane-associated Ig expressed during B cell ontogeny and secreted during Ag-specific immune responses. IgM can be divided into two types: circulating IgM that exists independent of known immune exposure, which are referred to as natural IgM (nIgM), and immune IgM, which is generated in response to defined antigenic stimuli. In healthy adults, circulating polyclonal IgM is generally present at 1–2 mg/ml in the blood. As the first line of defence against invading microbes or stress damage, polyclonal nIgM can directly recognize a wide range of pathogen-associated molecular patterns (PAMPs) on different microbial pathogens or damage-associated molecular patterns (DAMPs), such as ssDNA and dsDNA, which are released by damaged or necrotic tissues. nIgM usually displays polyreactivity and triggers a rapid humeral immune response (Deng et al., 2006; Zhu et al., 2008; Huang et al., 2008, 2009; Zheng et al., 2009; Zhang et al., 2010; Kimoto, 1998).

In this study, using liver and small intestine epithelial cells in adult Balb/c mice as models, we further revealed IgM expression and expression of its functional transcripts in epithelial cells that were sorted by flow cytometry. Moreover, we showed that IgM expression and secretion were significantly upregulated by CpG ODN, a synthetic analog of bacterial DNA. Importantly, we further identified the natural antibody properties of this IgM.

2. Results

2.1. IgM expression in the epithelial cells of Balb/c mice

We first determined whether IgM is present in epithelial cells, including epithelial cells from the pancreas, liver, lung, kidney, stomach, uterus and small intestine of mice. Immunohistochemistry studies showed that IgM was present in the cytoplasm of these epithelial cells (Fig. 1A). To further elucidate that IgM could be spontaneously expressed by this cell type, epithelial cells were isolated and sorted from the liver and small intestine of mice according to their cytokeratin (CK) expression profiles using flow cytometry (CK18 for liver epithelial cell, and CK8 for IECs) (Fig. 1B and D), and the IgM gene rearrangement and transcription levels were assessed. To exclude the possibility of B cell contamination, the sorted epithelial cells was evaluated for the absence of transcripts of CD19, CD20, and CD138. Then, specific primers were used to amplify the constant and variable regions of the Ig κ and μ chains, respectively. This analysis demonstrated the presence of rearranged Ig μ and Ig κ transcripts in liver epithelial cells and IECs (Fig. 1C and E). IgM was also detected in sorted liver epithelial cells and IECs with goat anti-mouse IgM by Western blot analysis (Fig. 1F). FACS staining with PE-conjugated goat anti-mouse IgM further confirmed the existence of the IgM protein (Fig. 1G).

2.2. Sequence analysis of the variable regions of the Ig μ and κ transcripts

The IgM gene transcripts and repertoires of the liver epithelial cells and IECs from six Balb/c mice were detected. Spleen cell-derived IgM gene transcripts served as the positive control. The sequencing results demonstrated that 30 sequences of 32 V_HDJ_H rearrangements and all of the 42 VκJκ rearrangements from the liver epithelial cells showed a functional and typical V(D)J rearrangement pattern. Additionally, nIgM from B-1 cells are often without N-region additions and are germline-encoded or with minimal somatic hypermutations. The sequence analysis revealed that although almost all of the liver epithelial cell-derived Ig μ transcripts had N-regions, all of the Ig κ transcripts showed no N-region additions. Moreover, both of the Ig μ and Ig κ transcripts possessed germline or near germline sequences. Different from the rearrangement diversity that was observed in the spleen cells, the liver epithelial cell-derived Ig μ variable regions showed conserved usage in different individuals (Table 1). $V_{IGHV1\text{-}37^*01}/D_{IGHD2\text{-}13^*01}/J_{IGHJ2^*01}$, the predominate VDJ rearrangement pattern in two of the five mice, was the preferential usage in the epithelial cells. Similarly, the rearrangement patterns of the Ig κ variable region in the liver epithelial cells revealed conserved usage in different individuals (Table 2). We also obtained the transcripts of the Ig κ variable region in the IECs, which showed similar characteristics to that in the liver epithelial cells (supplementary Table 1). $V_{IGKV9\text{-}124^*01}J_{IGKJ2^*01}$ recombination occurred in both the liver epithelial cells and the IECs but not in the spleen cells.

2.3. A TLR9 agonist stimulated epithelial cells to secrete IgM in mice

The TLR9 signaling pathway is an important regulator for IgM secretion in B cells. To determine whether IgM from epithelial cell has effects on bacterial invasion defence, the epithelial cell IgM expression levels were detected when mice were stimulated with the TLR9 agonist, CpG, which mimics bacterial infection. TLR9 expression in liver and small intestine epithelial cells was detected (data not shown) by IHC. Additionally, the TLR9 transcripts in these cells were further confirmed by RT-PCR (Fig. 2A). Subsequently, the TLR9 signaling pathway regulating IgM secretion in epithelial cells was activated carried out by the CpG treatment. As expected, the mouse serum IgM levels were significantly elevated (Fig. 2B). Importantly, the result revealed that the IgM transcription after CpG stimulation (but not with a GpC control or PBS) was significantly upregulated in flow cytometer-sorted liver epithelial cells and IECs (Fig. 2C–F). The Western blot results further revealed that the IgM levels in the liver and small intestine were increased after stimulation with CpG (Fig. 2H). Moreover, the CpG induction punctuated the accumulation of IgM in the extracellular matrix of the liver epithelial cells and in the small intestinal lumen (Fig. 2G). ELISPOT results further confirmed that the CpG treatment promoted IgM secretion of these epithelial cells (Fig. 2I).

2.4. Epithelial cell-derived IgM displayed natural IgM properties

The fact that IgM was spontaneously expressed in epithelial cells guided us to explore its potential functions as a natural antibody. Natural antibodies are often polyreactive and can bind to multiple antigens with low affinity. Natural antibody activities of IgM produced from several epithelial tissues of mice, including liver, pancreas, lung and small intestine, were analyzed. Using an ELISA assay, we found that, similar to the B-1 cell-derived nIgM, the epithelial cell-derived IgMs also showed polyreactivity compared to the non antigens-coating microwells, they could bind ssDNA, dsDNA, LPS, insulin as well as different types of microbes (Fig. 3Aa).