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CD9 may contribute to the survival of human germinal center B cells by facilitating the interaction with follicular dendritic cells

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ABSTRACT

The germinal center (GC) is a dynamic microenvironment where antigen (Ag)-activated B cells rapidly expand and differentiate, generating plasma cells (PC) that produce high-affinity antibodies. Precise regulation of survival and proliferation of Ag-activated B cells within the GC is crucial for humoral immune responses. The follicular dendritic cells (FDC) are the specialized stromal cells in the GC that prevent apoptosis of GC-B cells. Recently, we reported that human GC-B cells consist of CD9+ and CD9− populations and that it is the CD9+ cells that are committed to the PC lineage. In this study, we investigated the functional role of CD9 on GC-B cells. Tonsillar tissue section staining revealed that *in vivo* CD9+ GC-B cells localized in the light zone FDC area. Consistent this, *in vitro* CD9+ GC-B cells survived better than CD9− GC-B cells in the presence of HK cells, an FDC line, in a cell–cell contact-dependent manner. The frozen tonsillar tissue section binding assay showed that CD9+ GC-B cells bound to the GC area of tonsillar tissues significantly more than the CD9− GC-B cells did and that the binding was significantly inhibited by neutralizing anti-integrin β1 antibody. Furthermore, CD9+ cells bound to soluble VCAM-1 more than CD9− cells did, resulting in activation and stabilization of the active epitope of integrin β1. All together, our data suggest that CD9 on GC-B cells contributes to survival by strengthening their binding to FDC through the VLA4/VCAM-1 axis.

1. Introduction

Tetraspanins are a large superfamily of proteins with four highly conserved transmembrane domains [1]. Tetraspanins interact with one another and many other proteins, organize a network of molecular interactions into functional microdomains, and regulate cellular process in which the associated proteins are involved [2,3]. The list of tetraspanin-associated proteins is growing and includes integrins and other adhesion molecules, proteins with Immunoglobulin (Ig) domains, enzymes (e.g., ectopeptidases and metalloproteases), and intracellular signaling molecules (e.g., heterotrimeric G proteins, PI4K, activated PKC) [4]. In immune cells, tetraspanins interact with key leukocyte receptors such as the B cell receptor (BCR) complex, CD4/CD8, MHC molecules, and integrins, and thus modulate leukocyte receptor activation and downstream signaling pathways [5]. Recent studies with mice lacking CD37 and CD81 showed that these tetraspanins on B cells are important in the humoral immune response [5]. CD37 on B cell supports long-lived plasma cell survival by orchestrating the VLA4-AKT signaling axis [6], while CD81 on B cells organizes CD19, which is required for the effective BCR signaling [7]. In contrast, the tetraspanins CD9, CD151, and Tssc6 appeared to be dispensable in the B cell immune response [5]. The functions of tetraspanins in the human B cell-mediated immune response, however, have not been largely explored yet. Recently, we reported that CD9 is expressed in a subset of the human tonsillar germinal center (GC) B cells but not in naïve and memory B cells [8]. The specific expression of CD9 on human GC-B cells in contrast to mouse GC-B cells that do not express CD9 [9] suggests that CD9 on human GC-B cells may have a functional role in the GC microenvironment.

The GC is a specialized microenvironment where high-affinity antibody-secreting plasma cells (PC) and memory B cells are generated. Recently introduced imaging approaches advanced our understanding of the germinal center as a highly dynamic structure in which B cells move around and interact with other neighboring cells to produce B cells with high affinity [10,11]. Following the BCR stimulation by antigens, B cells migrate into the GC and rapidly proliferate within the GC microenvironment

Abbreviations: Ag, antigen; BCR, B cell receptor; GC, germinal center; FDC, follicular dendritic cells; PC, plasma cells

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created by follicular dendritic cells (FDC) and T cells. During this phase of rapid proliferation, antibody diversification processes such as somatic hypermutation and class-switch recombination take place. Somatic hypermutation introduces point mutations into the IgV gene and class-switch recombination alters the effector function of the antibody by switching the constant region of IgM to the IgG, IgE, or IgA isotypes. Subsequently, newly mutated cells expressing high-affinity BCR against antigen on FDC are selected and subsequently present this antigen to T cells for further selection. During the selection process by BCR, the B cells are programmed to die unless survival signals are provided from FDC [12]. While several adhesion molecules and soluble factors from FDC have been reported as promoting GC-B cell survival [13], physical interactions appeared to be necessary for FDC-mediated GC-B cell survival [14]. Adhesion molecules such as ICAM-1 and VCAM-1 on FDC mediate the anti-apoptotic FDC effect on GC-B cells [15].

Here, we investigated the functional role of CD9 on B cells within the GC microenvironment using human tonsillar GC-B cells, which consists of CD9− and CD9+ populations [8]. CD9 expression on human GC-B cells enhances the affinity of VLA4 to VCAM-1 on FDC. The strong adhesion of GC-B cells to FDC is promoted by CD9, which in turn contributes to better survival of GC-B cells.

2. Results

2.1. CD9+ GC-B cells reside in the FDC area of the light zone of the GC

The interactions between GC-B cells and FDC are essential for GC-B cell survival and differentiation [13]. Since CD9 is implicated in the cell–cell interaction [16–18], we hypothesized that CD9 on GC-B cells may play a role in the interaction between GC-B cells and FDC. To address this question, we first determined whether CD9+ GC-B cells were located in the FDC area *in vivo*. Previously, we demonstrated that tonsillar GC-B cells were composed of CD9+ and CD9− populations and that this ratio was various from donor to donor [8]. Consistent with our previous observation, a portion of CD10+ GC-B cells expressed CD9 when tonsillar tissue sections were stained with anti-CD10 and anti-CD9 antibodies (Supplemental Fig. 1). The tonsillar tissue sections were further stained with antibodies against FDC markers in combination with anti-CD9 to determine whether CD9+ GC-B cells were situated in the FDC area (Fig. 1A and B). Among FDC markers, CD23 and CD54 were selected because CD23+ FDC and CD54+ FDC reside in the light zone of the GC, where FDC are abundant [19]. CD9+ cells were localized both in the apical and basal light zones of the GC where CD23+ and CD54+ FDC, respectively, reside [20]. Furthermore, the majority of CD9+ cells did not express Ki-67, a marker for centroblasts of the dark zone area (Fig. 1C). Although CD9+ and Ki-67+ cells were not mutually exclusive, the staining pattern showed that CD9+ cells and Ki-67+ cells occupied two distinctive areas, confirming that CD9+ cells were located in the light zone of the GC. The stainings were specific because no expression was observed in tonsillar tissue sections stained with corresponding control antibodies (data not shown). These data suggest that CD9+ GC-B cells are in close contact with FDC *in vivo*.

2.2. CD9+ GC-B cells survive better than CD9− GC-B cells in the presence of FDC/HK cells in a cell–cell contact-dependent manner

The *in vivo* location of CD9+ GC-B cells in the FDC area prompted us to determine whether CD9 has a functional role in

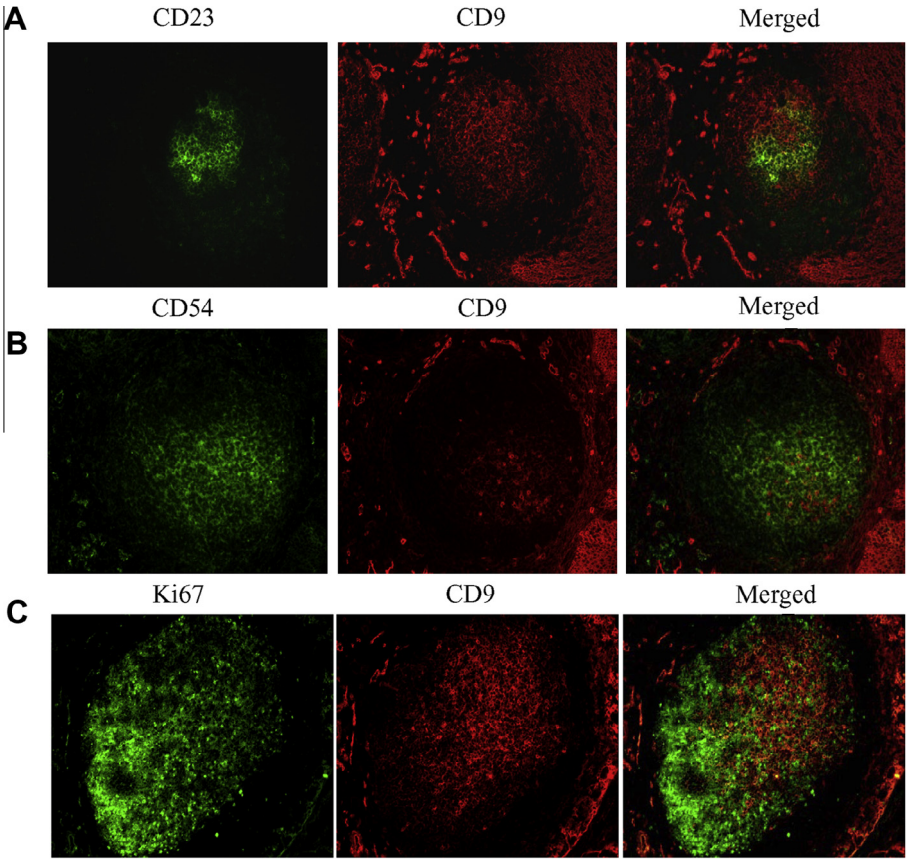

Fig. 1. Immunofluorescent staining for CD9 in the germinal centers of human tonsils. The frozen tonsillar tissue sections were stained with anti-CD9 (A–C, red) in combination with anti-CD23 (A, green), anti-CD54 (B, green), and anti-Ki67 (C, green). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)