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Research Article

Macrophages enhance tumor-derived autophagosomes (DRibbles)-induced B cells activation by TLR4/MyD88 and CD40/CD40L

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ABSTRACT

Our previous studies have showed that tumor-derived autophagosomes (termed "DRibbles") induce B cell activation, resulting in antibody production and cytokine secretion. Unexpectedly, we found that unfractionated splenocytes produced a higher level of antibody and cytokine than that of purified B cells. In the current study, we investigated the role of accessory cells in DRibbles-induced B cell activation. We found that cognate macrophages, but not T cells, significantly enhanced the B cell activities. Such an enhancement required cell–cell contact. Furthermore, DRibbles stimulation up-regulated CD40L expression on macrophages, resulting in increased level of CD40 expressed on B cells. The accessory role of macrophages in DRibbles-activated B cells is critically dependent on the CD40/CD40L interaction. In addition, the effects of macrophages were found to be largely dependent on TLR4 and MyD88 signaling pathway. Finally, our results showed that macrophages were able to enhance the antigen presentation function of B cells for specific T cell stimulation. Thus, these results suggest that macrophages play an important accessory role for DRibbles-induced B cell immune function.

Abbreviations: WT, wide-type; Mφ, macrophage; BAFF, B cell activating factor; TNF, Tumor Necrosis Factor; DRibbles, tumor-derived autophagosomes; DC, dendritic cell; APC, antigen-presenting cells; TGF-β, transforming growth factor-β; TLRs, Toll-like receptors; BMDM, Bone marrow-derived macrophages.

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Introduction

Tumor-derived autophagosomes, also termed DRibbles, sequestered a broad range of tumor antigens. They could be cross-presented by dendritic cells (DC) to prime antigen-specific T cells [1]. In addition to DCs, our previous studies have demonstrated that B cells can also be activated by DRibbles, resulting in antibody production, cytokine secretion in a TLR2/MyD88 modulated manner. Moreover, DRibbles-loaded B cells could act as antigen-presenting cells (APC) for the stimulating specific T cells, a process shown to be independent of DCs [2].

As a key component of the adaptive immunity, B cells have been found to encounter small soluble antigens and large particulate antigens that are attached to the surface of neighboring cells, such as follicular dendritic cells (FDCs), or macrophages [3–6]. Following crosslink with antigens, B cells can proliferate and differentiate into antibody secretion cells. They are efficient antigen-presenting and cytokine producer cells. These functional developments occur only after receiving contact-dependent help delivered by the interaction with CD40L on activated follicular T helper cells [7]. The CD40/CD40L interaction is essential for B-cell survival, formation of germinal centers, generation of memory-cell populations, and somatic hypermutation [8,9]. Furthermore, growing evidence shows that macrophages, dendritic cells and other cells of the innate immune system provide the necessary signals for full activation and differentiation of B cells [10].

Macrophages are important accessory cells for generation of high specific adaptive immune responses and crucial mediators of the innate immune response. In addition to its primary function, macrophage-derived signals have long been known as being indispensable for B cell activation in vitro [11,12]. Moreover, macrophages can directly regulate B-cell proliferation via the TNF ligand, BAFF (B cell-activating factor belonging to the TNF family), and support the idea that they may play an important accessory role during TI immune responses [13]. In addition, B cells can serve as recipients of antigen transferred from macrophages within the subcapsular sinus of the lymph node in the form of immune complexes or large particulate Ag [5,14].

In this study, we sought to investigate the accessory role of cognate macrophages in the activation of B cells. We showed that macrophages directly enhanced the activation of B cells induced by DRibbles via CD40/CD40L. This process did not require soluble molecules. The accessory functions of macrophages induced by DRibbles were found to be largely dependent on TLR4 and MyD88 signaling pathway. Finally, we demonstrated that DRibbles-activated B cells were highly efficient in inducing T cell immune responses in vitro when activated in the presence of cognate macrophages.

Materials and methods

Mice, cell lines and reagents

C57BL/6 mice, MyD88$^{-/-}$, TLR4$^{-/-}$ and TLR2$^{-/-}$ mice were purchased from the Model Animal Research Centre of Nanjing University (Nanjing, China). All mice were bred and maintained in a specific pathogen-free condition. All experimental protocols were approved by the Institutional Animal Care and Use Committee of Southeast University. E.G7-OVA cells were cultured in complete medium made of RPMI 1640 (Life Technologies, Carlsbad, CA), supplemented with 10% heat-inactivated FCS (Life Technologies), 100 U/ml penicillin, 0.1 mg/ml streptomycin (Beyotime Institute of Biotechnology, Haimen, China).

DRibbles isolation

DRibbles were prepared from E.G7-OVA cells as described previously [15]. Endotoxin contamination in DRibbles was determined by using the Limulus Amebocyte Lysate QCL-1000 assay (Cambrex, Walkersville, MD, USA), and the endotoxin level of DRibbles was <0.2 EU/mg. The total amount of protein in DRibbles was quantified by BCA protein assay Kit according to the manufacturer's protocol (Beyotime Institute of Biotechnology).

B cells, T cells preparation

Splenic B cells were purified by negative selection using the magnetic microbeads (Life Technologies). Magnetic microbeads, which coated with streptavidin and conjugated to a biotinylated anti-mouse CD43 antibody, were added directly to the sample and bound the CD43$^+$ cells. The unbounded-cells are quickly separated by the magnet, in which CD19$^+$ cells were confirmed to be $\geq 95\%$ by FACS. Dynabeads Mouse pan T kits (Life Technologies) were used to isolate or deplete mouse T-cells.

Macrophage preparation

Bone marrow-derived macrophages (BMDM) from wild-type, TLR2$^{-/-}$, TLR4$^{-/-}$ and MyD88$^{-/-}$ mice were generated as previously described using mouse macrophage colony-stimulating factor (Shenandoah Biotech) [16]. Briefly, single-cell suspensions of bone marrow were prepared from the femurs of C57BL/6 mice, and then cultured in RPMI 1640 (Life Technologies) supplemented with 10% FCS (Life Technologies), 100 U/ml penicillin, 100 g/mL streptomycin for 7 days in the presence of macrophage-colony-stimulating factor (20 ng/ml). In some experiments, splenic macrophages with a CD11b$^+$ F4/80$^+$ Gr1$^-$ phenotype were depleted by the cell sorter (FACStar^{Plus}, BD Biosciences). Fixation of BMDM was carried out by incubation with 4% paraformaldehyde for 10 min at 4 °C after 6 h-incubation with DRibbles. Subsequently, BMDM were washed three times with PBS before using.

The activation of B cells or macrophages induced by DRibbles

Unfractionated splenocytes, purified B cells or BMDM (10^6/ml/well) were stimulated with DRibbles (10 μg/ml total proteins), whole tumor cell lysate (10 μg/ml total proteins) or LPS (10 μg/ml) for 72 h. Cell culture supernatants were collected for quantitation of IgM, IL-6 and IL-10. BMDM were collected and analyzed the expression of co-stimulatory molecules by FACS. In some experiment, wide-type BMDM were pre-incubated with TLR4 inhibitor-TAK-242 (MCE, Monmouth Junction, New Jersey) for 1 h, washed with PBS for 3 times, and then stimulated subsequently with DRibbles for 72 h.

Coculture of B cells with macrophages or T cells

B cells were cocultured with BMDM (at ratio of 3:1) or T cells (at ratio of 1:1) in 24-well plates. For Transwell (Millicell, 1.0 μm; Millipore) experiments, BMDM (1×10^6) in a volume of 1.5 ml