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Transfer of antigen from human B cells to dendritic cells

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ABSTRACT

The cooperation of B lymphocytes with other antigen presenting cells (APCs) is often necessary in the efficient processing and presentation of antigen. Herein, we describe a mechanism by which B cells physically interact with dendritic cells (DCs) resulting in the transfer of B cell receptor (BCR)-enriched antigen to these APCs. Antigen transfer involves direct contact between the two cells followed by the capture of B cell derived membrane and intracellular components. Strikingly, DCs acquire greater amounts of antigen by transfer from B cells than by endocytosis of free antigen. Blocking scavenger receptor A, a DC surface receptor involved in membrane acquisition, abrogates these events. We propose that antigen transfer from B cells to DCs results in a more focused immunologic response due to the selective editing of Ag by the BCR.

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1. Introduction

Antigen presentation plays a critical role in directing immune responses to a variety of foreign antigens and under pathological conditions, to self-antigens. It is the requisite function of several different cell types of the immune system, notably macrophages, dendritic cells (DCs), and B cells, to capture, process and present antigens. Individually, these antigen presenting cells (APCs) have been shown to induce T cell responses, whether immunogenic or tolerogenic, depending on the context in which the antigen is presented (Crowley et al., 1990; Inaba et al., 1990; Jong et al., 2006; Lanzavecchia, 1990; Mannhalter et al., 1983; Roth et al., 1997). Indeed, autologous DC based therapies have become a part of the landscape of tumor treatment. However, the cooperation and/or direct interaction between similar or different types of APCs can promote a more effective and robust immune response (Balázs et al., 2002; Diaz-de-Durana et al., 2006; Kleindienst and Brocker, 2005; Wykes et al., 1998; Yan et al., 2006). For instance, antigen specific T cell activation and expansion is much greater in transgenic mice that selectively expressed the T cell restricting MHC II molecule on both B cells and dendritic cells as compared to transgenic mice that expressed the MHC II in a single APC population (Kleindienst and Brocker, 2005). Mice individually deficient in B cell APCs, macrophage or DCs do not activate T cells to the same magnitude observed in wild type hosts (Chan and Shlomchik, 1998; Duffield et al., 2005; Probst and Broek, 2005).

We have previously demonstrated that antigen specific B cells can induce a T cell response to soluble antigen earlier than other APCs, due to their ability to rapidly capture antigen efficiently through the B cell receptor (BCR) (Lanzavecchia, 1990; Pape et al., 2007; Yan et al., 2006). However, the B cell mediated APC function is short-lived. Long-lived APC functions are best performed by DCs indicating that cooperation between these two APCs is necessary to achieve a productive immune response. Our studies and that of Kleindienst and Brocker highlight the importance of the collaboration between DCs and B cells in immunity (Kleindienst and Brocker, 2005; Yan et al., 2006). Both of these APCs have been shown to modulate the function of the other through cytokine production and antigen presentation (Bayry et al., 2005; Dubois et al., 2001; Kilmon et al., 2005). Furthermore, the direct interaction of B cells with DCs has been clearly demonstrated *in vitro* as well as *in vivo* (Huang et al., 2005; Qi et al., 2006). In both of these studies, antigen bearing DCs contact and activate antigen specific B cells. Additional studies have illustrated that DCs can provide antigen directly to B cells by unknown pathways (Balázs et al., 2002; Bergtold et al., 2005; Wykes et al., 1998). Conversely, several studies have implied that the reverse may also occur in that B cells can transfer antigen

Abbreviations: MoDC, myeloid DC; MdDC, monocyte derived DC; alg, anti-human IgG/M F(ab′)2; SR-A, class A scavenger receptor; IC, immune complex; B-LCL, B lymphoblastoid cell line; CTO, CellTracker™ Orange; CTFR, CellTrace™ Far Red; AF488, Alexa Fluor® 488; TW, transwell.

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to DCs (Ferguson et al., 2004; Valdez et al., 2002); however, direct evidence of this pathway has been lacking.

Previously, we have shown using fluorescently labeled antigen that antigen specific B cells can transfer antigen to macrophages and that this process can activate a T cell response both *in vitro* and *in vivo* (Harvey et al., 2007, 2008). Here we demonstrate that human B cells can transfer BCR-targeted antigen to human DCs and that direct interaction between the two APCs is necessary for this event to occur. The predominant mechanism of antigen transfer described herein involves the capture of B cell derived membrane and/or intracellular proteins by the recipient DCs in a process known as trogocytosis. Furthermore, we have identified scavenger receptor A as a key surface receptor on the human DCs that mediates the exchange of cell membrane components along with BCR-enriched antigen. Recipient DCs appear to carry processed forms of antigen. Therefore, antigen transfer could enable the presentation of antigen to T cells by the DCs and thus, induce an immunologic response. We propose that BCR-mediated sequestration and subsequent transfer of specific antigens to other APCs such as DCs leads to a more focused immune response by discriminating a particular set of antigens from a diverse array of potential targets.

2. Materials and methods

2.1. Isolation and tissue culturing of cells

Human PBMCs were isolated from leukopacks (New York Blood Center, Long Island City, NY) by the Ficoll-Hypaque method previously described (Bennett and Cohn, 1966). Lineage marker specific cells (Lin1$^+$: CD3, CD14, CD16, CD19 and CD56) were separated from DCs by positive selection using magnetic beads (StemCell Technologies). The negatively selected population was stained with Lin1-FITC, anti-HLA-DR-PE, CD11c-PECy5 (BD Pharmingen) and CD123-APC (Miltenyi Biotech) antibodies and sorted on a FacsAria (Becton Dickinson) for HLA-DR$^+$:CD11c$^+$:CD123$^-$ primary myeloid DCs (MoDCs). MoDCs were cultured in RPMI with 10% heat-inactivated human male AB sera (Sigma) and used immediately. Human monocyte derived DCs (MdDCs: StemCell Technologies) were cultured in the same medium as above with addition of 50 ng/ml recombinant human GM-CSF and IL-4 (R&D Systems) for 24 h prior to use. Primary human B cells were isolated from PBMC by negative selection using magnetic beads (StemCell Technologies) and cultured in same medium as DCs. Human B cell lines B-LCL and BJAB were maintained in 10% FBS RPMI 1640 medium.

2.2. Preparation of fluorescent antigen

Anti-human IgG/IgM F(ab′)2 antibody fragments (aIg; Jackson ImmunoResearch Laboratories) were conjugated with Alexa Fluor® 488 (AF488; Molecular Probes) at a 1:6 molar ratio, respectively, using the succinimidyl ester form. Antibody was separated from unreacted fluorophore by centrifugation through concentrator (Millipore) and resuspended in PBS. The double conjugated antigen of aIg with AF488 and the pH-sensitive fluorogenic dye pHrodo™ (Molecular Probes) (aIg-AF488/pHrodo) was generated as above with molar ratio of 1:3:3, respectively.

2.3. Uptake of antigen by B lymphocytes

B-LCL or BJAB cells were cultured for 15 min in the presence of 10% human serum RPMI 1640 medium and 1 mg/ml human Ig (Sigma) to block Fc receptors. Cells were washed twice in pre-warmed HBSS and once in 10% FBS RPMI medium to remove excess Ig. For various time points, B cells (2×10^7 cells/ml) were pulsed with 10 μg/ml of either aIg or anti-FITC Ig conjugated with AF488 (non-specific antibody; Molecular Probes) at 37 °C/5% CO_2 followed by 4 washes with ice-cold HBSS and a wash with 10% human serum RPMI 1640 medium. Level of antigen uptake was determined by fluorescence microscopy of wet mounts and by flow cytometry after anti-CD19-PE (BD Pharmingen) staining. Optimal incubation time of B cells with antigen was found to be 60 min. Primary human B cells were pulsed with antigen as described except the Fc receptor-blocking step was omitted.

2.4. Antigen transfer assays with human dendritic cells

Dendritic cells (1×10^6 cells/well) were co-cultured for 18 h with B cells (2×10^6 cells/well) that had been pulsed with one of the following: no antigen, non-specific antibody or aIg. All cells were harvested and then stained for flow cytometry with anti-CD11c-PECy5 (for DCs) as well as biotinylated anti-CD19 (BD Pharmingen) and anti-CD14 (Invitrogen-Caltag) antibodies followed by streptavidin-AF647 to identify B cells and monocytes (contaminating population), respectively. Level of antigen (AF488$^+$) was evaluated for the CD19$^-$:CD14$^-$:CD11c$^+$ gated population that was derived from non-B lymphocyte FSC:SSC gated cells. With co-cultures of DCs and primary B cells, antigen levels were assessed for the same gated population as above except this gate was derived from the FSC:SSC gate that included both cell types, since these cells are nearly equivalent in size. For co-cultures with soluble antigen, DCs were cultured for 18 h under the following conditions: no antigen, 5 μg/ml soluble aIg (no B cells), or BJAB cells pulsed with either non-specific Ab or aIg.

2.5. Live cell imaging of antigen transfer by confocal microscopy

MoDCs (1×10^6 cells per dish) were cultured in glass bottom (1.5 mm thickness) dishes (Mattek) 24 h prior to use. Both MdDCs and primary MoDCs were labeled with 0.5 μM CellTrace™ Far Red (CTFR) (DDAO-SE; Molecular Probes) for 8 min in HBSS (no phenol red) and the reaction stopped with 5-fold excess of FBS. Cells were then washed twice in HBSS and resuspended in culture medium. BJAB cells were labeled with 5 μM CellTracker™ Orange (CTO) (CMRA; Molecular Probes) and pulsed with antigen. Each DC population (1×10^6 cells) was co-cultured separately with antigen pulsed B cells (2×10^6) in glass bottom dishes. After 18 h, live cell cultures were examined by confocal microscopy (LSM510meta; Zeiss) at 600× magnification and 2× zoom.

2.6. Assay for antigen transfer from apoptotic B cells

BJAB cells (2×10^6 cells) or primary B cells were pulsed with antigen and induced to undergo apoptosis by hyperthermia (30 min at 65 °C) as previously described (Zhao et al., 2006). Induction of apoptosis was confirmed by flow cytometry using the vital dye TO-PRO-3 (Molecular Probes). Given that 15% of BJAB cells (3×10^5 cells of 2×10^6 cells) undergo apoptosis/necrosis when cultured alone (B. Harvey and M. Mamula, unpublished data), primary MoDCs were co-cultured with 3×10^5 apoptotic or 2×10^6 viable B cells for 18 h prior to being harvested.

2.7. Preventing cell contact between antigen donor and recipient by transwell

Primary DCs (1×10^6 cells/well) were seeded into a 24 well plate. Antigen pulsed B cells were added to transwells (1, 3, or 8 μm; BD Falcon) above the wells containing DCs and cultured for 18 h. Cells in the transwells were collected and analyzed by flow cytometry separately from those within plate wells.

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