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Research paper

T cell hybridomas to study MHC-II restricted B-cell receptor-mediated antigen presentation by human B cells

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ABSTRACT

MHC-II antigen presentation by B cells is essential in order for B cells to receive optimal costimulation from helper CD4+ T cells. This process is facilitated and focused through the extremely efficient uptake, processing, and presentation of antigen recognized by an individual B cell's unique B-cell receptor (BCR). The investigation of human B-cell antigen presentation has been limited by the varied specificity of BCR found in the mixed populations of B cells *in vivo*. As a result, there is no readily available method to measure BCR-mediated antigen presentation in this heterogeneous population of B cells. We have overcome this limitation by developing HLA-DR-restricted T-cell lines capable of recognizing a specific antigen taken up via the BCR and presented by the mixed B-cell population through this physiologically relevant mechanism. BCR-mediated presentation was enhanced >4 logs compared to presentation by B cells taking up the antigen through nonspecific mechanisms. The studies presented here characterize T-cell hybridoma lines developed for HLA-DRB1*0101+ and HLA-DRB1*1501+ B cells, but clones could be generated for other HLA-DR types using the methods described. These hybridomas have potential applications including study of the mechanisms of BCR-mediated enhancement of presentation, determination of adjuvant effects on presentation, and optimization of vaccine antigen preparations. Therefore, these T-cell lines could significantly facilitate the study of BCR-mediated antigen presentation required by T helper cell-dependent vaccines in humans.

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1. Introduction

Future investigations into the mechanism of B-cell antigen presentation can provide greater understanding of the human adaptive immune response to disease, as well as insights for effective vaccine development. This provides a compelling rationale for the development of more effective and practical tools to further these scientific endeavors.

For T-cell dependent antigens, naive B cells, on encountering the specific antigen that their BCR recognizes, initiate a series of interactions with CD4+ helper T cells leading to proliferation and differentiation into plasma cells and memory B cells. The involvement of the BCR as a critical component of B-cell antigen presentation has been well described (Chesnut and Grey, 1981; Lanzavecchia, 1990). B cells are >1000 fold more effective at presenting antigen that can bind to the BCR and are taken up through that mechanism rather than nonspecific mechanisms (Rock et al., 1984; Lanzavecchia, 1985). The determination soon followed that B-cell antigen presentation demands BCR specificity for antigen, processing by the internal machinery of the B cell, and MHC-restricted presentation on the cell surface (Lanzavecchia and Bove, 1985). The exquisite specificity for B-cell antigen presentation provides an effective mechanism for the selective delivery of T-cell help required for optimal B-cell function for T helper-dependent antigens.

B-cell proliferation, activation and antibody class switching are dependent on interaction with CD4+ helper T cells

Abbreviations: APC, antigen presenting cell; BCR, B cell receptor; TCR, T-cell receptor; RT, reverse transcriptase; HAT, hypoxanthine/aminopterin/thymadine.

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(Mitchison, 1971). There are data that differences in the binding strength of the T-cell receptor (TCR) and B cell in the antigen-specific interaction help program specialized follicular T helper cells to develop an effector function *in vivo* (Fazilleau et al., 2009). This provides a rationale for the importance for understanding the antigen presentation function of B cells in human systems. Increased understanding of MHC-II antigen presentation could result in development of adjuvants or optimization of antigen delivery for improved vaccines.

Fusion of B cells to an immortal fusion partner was developed in the 1970s to generate B-cell hybridomas that secrete monoclonal antibodies (Kohler and Milstein, 1975). Shortly thereafter, this technique was applied to T cells to produce T-cell hybridomas that secrete IL-2 after TCR signaling (Kappler et al., 1982; Rock et al., 1990). In light of the practical advantages of using peptide-specific T-cell hybridomas, investigators have widely adopted them as a tool to quantitatively measure peptide-specific antigen presentation by multiple types of antigen presenting cells (APC). Vidovic et al. demonstrated that the adhesion molecules and integrins in human and murine T cells are very highly functionally conserved and murine T-cell:human APC interaction occurred readily (Vidovic et al., 2003). We and others have used HLA-DR transgenic mice to make T-cell hybridomas that respond readily to human APC (Woods et al., 1994; Canaday et al., 2003; Gehring et al., 2003; Vidovic et al., 2003). T-cell hybridomas have a number of advantages over T-cell lines in the study of APC function. They can be generated to specific antigen or epitopes, are reliable, reproducible and convenient to use. They can be grown to unlimited supply for large antigen presentation experiments as well.

B cells in blood and tissues necessarily have BCR specificities for a wide variety of potential antigens. As a consequence, the investigation of BCR-mediated antigen presentation of any one specific antigen is difficult in this mixed population of cells. We have made use of anti-Ig (anti-BCR) antibodies to not only target the BCR and be taken up via this receptor, but also to serve as the presented antigen that is then recognized by the T cell. This technique has been used in animal systems with success (Chesnut and Grey, 1981; Gosselin et al., 1988). By circumventing the need to isolate B cells of a known specificity, the use of anti-human BCR as antigen allows for the study of BCR-mediated antigen presentation with readily available quantities of primary human B-cells.

We have set out with the goal of developing a T-cell hybridoma system that is adapted to the study of antigen presentation in human B cells. This system requires that the antigen be taken up by the global population of BCR-expressing B cells and then be recognized by the T-cell hybridoma. In this paper, we characterize the HLA-DR restricted T-cell hybridomas we developed to study BCR-mediated antigen presentation in primary human B cells.

2. Materials and methods

2.1. Cell lines, mice, antigens and inhibitors

HLA-DRB1*0101 transgenic mice were obtained from Dennis Zaller (Merck Laboratories, Whitehouse Station, NJ) (Rosloniec et al., 1997) and the HLA-DRB1*1501 transgenic mouse from Chella David (Mayo Clinic).

HLA-DR1+ and HLA-DR15+ EBV-lymphoblastic cell lines were used. HLA-DRB1*0101-restricted T cell hybridoma specific for HIV reverse transcriptase (RT) were previously generated and described (Jones et al., 2007). BW1100, a variant of BW5147 that does not express TCR, was used (Born et al., 1988). Standard media for EBV-lymphoblastic cell lines was RPMI (Cambrex, East Rutherford, NJ) with 10% fetal calf serum.

Goat anti-human IgM was purchased from Lampire (Pipersville, PA). Purified goat Fab, Fc fragments, and goat anti-human IgM were purchased from Invitrogen (Carlsbad, CA). Rabbit IgG and Fab anti-human IgM were purchased from Jackson Immunoresearch (West Grove, PA). We will refer to this anti-human IgM antibody as anti-BCR antibody for clarity in the rest of the manuscript. Rabbit serum was purchased from Zymed (Invitrogen). Anti-human HLA-DR antibody (L243) was purchased from BD Biosciences. Anti-human CD80 and anti-human CD86 were purchased from Biolegend (San Diego, CA). *Escherichia coli* recombinant Reverse Transcriptase (RT) was generated in our laboratory.

2.2. Generation of human antigen presenting cells

The human subjects protocol was approved by the Institutional Review Committee at University Hospitals and Case Western Reserve University and informed consent was obtained from all donors. PBMC were purified by Ficoll (GE Healthcare, Piscataway, NJ) per the manufacturer's instructions. B cells were purified in two ways. One method used immunomagnetic microbeads in the CD19 positive selection kit according to the manufacture's instructions (Miltenyi, Auburn, CA). The CD19+ B cells then underwent a second step of CD14 negative selection using the larger immunomagnetic beads (Dynal, Invitrogen) to remove any CD14+ monocyte contaminants. In the experiments where generation of dendritic cells was necessary, CD14+ monocytes were purified by positive selection (Miltenyi) first, then the flow through underwent CD19 positive selection (Miltenyi). Monocyte-derived dendritic cells were generated from CD14+ monocytes using IL-4 and GM-CSF as previously described (Canaday et al., 2003).

Purity of B-cells was determined by flow cytometry. Purified B-cells were incubated with fluorochrome-conjugated anti-DR, CD14, and CD19 antibodies for 10 min at room temperature, washed and fixed with 2% paraformaldehyde. Cells were analyzed on a FACS Calabur (BDBiosciences, San Jose, CA) flow cytometer. Cells expressing both HLA-DR and CD14 were considered to be monocytes. Purified B-lymphocytes had <5% CD14+ or DR+ CD19− cell contamination.

2.3. HLA-screening

DNA from donor's blood was purified by DNeasy kit (Qiagen, Valencia, CA) according to the manufacturer's instructions. Low resolution PCR screening for HLA-DR1 and DR15 was performed according to the manufacturer's instructions (Biosynthesis, Lewisville, TX). HLA-DR subtyping was confirmed by high-resolution kits (Biosynthesis).

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