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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.

Keywords

B cells; Antigen presentation; B cell receptor; MHC class II

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.

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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 (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 helps 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 & 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). *E. 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 (Dyna, Invitrogen) to remove any CD14+ monocyte contaminants. In the experiments where generation of dendritic cells was necessary, CD14+ monocytes were purified first 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 minutes at room temperature, washed and fixed with 2% paraformaldehyde. Cells were analyzed on a FACS Calibur (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).

2.4 T-cell hybridoma production and screening

HLA-DR1- and DR15-restricted T-hybridoma cell lines were generated according to previously established protocols (Canaday et al., 2003). Briefly, using Institutional Animal

Care and Use Committee approved protocols an emulsion of complete Freund's Adjuvant (Gibco-Life Technologies, Invitrogen) and 20 µg of goat IgG was injected in the footpad of the HLA-DR transgenic mice. After 7 days draining lymph nodes were harvested and cells restimulated with goat IgG *in vitro*. After 5 days cells were fused with BW1100 and grown in HAT selection medium. Goat-specific T-cells were identified by screening on human HLA-DR-matched EBV-lymphoblasts. Standard media for T-hybridoma cell lines and assays was DMEM plus 10% fetal calf serum with supplements as previously described (Canaday et al., 2003).

2.5. Antigen presentation assay with T-hybridoma cells

APC (2×10^4 – 5×10^4 /well unless otherwise specified) and T-hybridoma cells (1×10^5 /well) were plated in 96-well flat-bottom plates. When applicable, inhibitors to antigen presentation were added prior to the addition of antigen. APC and T-hybridoma cells were incubated with antigen for 22–24 h and supernatants harvested and frozen. CTLL-2 cells (5×10^3 /well) were added to thawed supernatants, followed by 15 µL of Alamar Blue (Trek Diagnostics, Westlake, OH) the next day. After overnight incubation, plates were read with a plate reading spectrophotometer at [O.D. 550 – O.D. 595]. All wells were plated in triplicate.

3. Results

3.1. Generation and characterization of HLA-DR1 restricted T-hybridoma cell lines

T-cell hybridomas were used as measures of specific peptide–MHC complexes. They secrete IL-2 in proportion to the number of specific peptide–MHC complexes on the APC. In these studies, IL-2 levels were determined with a bioassay that measures proliferation of the IL-2 dependent CTLL-2 line. Alamar blue, a dye indicating mitochondrial activity, is a non-radioactive method to assess CTLL-2 proliferation and thus peptide-specific MHC complexes presented to the hybridomas (Ahmed et al., 1994; Zhi-Jun et al., 1997; Kwack and Lynch, 2000).

Our experimental system was constructed so that the antigen recognized by the T-cell hybridoma is a goat antibody specific for human IgM, which is the BCR on the vast majority of B cells in the blood. This allows the antigen to be taken up by the BCR in the physiologic way that a B cell would take up antigen *in vivo*.

The first step in characterizing T-hybridoma cell lines was to demonstrate epitope specificity. T-cell hybridoma 1C was generated and screened for specificity to goat antibody. Fig. 1 shows responses of the 1C hybridoma to antigen presentation by dendritic cells of intact goat IgG with comparison to both Fab and Fc fragments of goat antibody. Hybridoma 1C responded to both IgG and Fab fragment demonstrating the specificity to goat Fab portion of IgG. The hybridoma does not recognize rabbit, bovine, or human IgG (data not shown).

Additional experiments were conducted to demonstrate antigen presentation is HLA-DR restricted. Fig. 2A illustrates the massive inhibition of antigen presentation of goat anti-BCR by human B cells with the addition of anti-HLA-DR antibody. Typical of most T-cell hybridomas, 1C is not costimulation dependent. The addition of anti-CD80 and anti-CD86 antibodies demonstrated minimal effect on the hybridoma response (Fig. 2B).

3.2. BCR-enhanced antigen presentation by primary human B cells

It is well known that BCR-mediated uptake of antigen enhances MHC-II presentation by B cells. Fig. 3A demonstrates the presentation by primary B cells to hybridoma 1C of goat

anti-BCR antibody compared to non-specific goat IgG. Anti-BCR specificity enhances antigen presentation by 3–5 orders of magnitude. Fig. 3B demonstrates that BCR-mediated antigen presentation by EBV-lymphoblasts was likewise significantly enhanced compared to presentation of non-specific goat IgG control. This establishes EBV-lymphoblast cell lines as an alternative to primary B cells as APC for use with this T-hybridoma.

3.3. Specificity of BCR-mediated enhancement of antigen presentation

We sought to confirm that enhanced presentation by B cells was dependent on BCR-mediated uptake of antigen rather than non-specific uptake mechanisms. These experiments used a rabbit anti-BCR that is not recognized by the T-cell hybridoma to serve as a blocking antibody for uptake of the goat anti-BCR, which is the antigen. Fig. 4 demonstrates concentration-dependent blocking of antigen presentation of goat anti-BCR by rabbit IgG and Fab anti-BCR. High concentration rabbit serum served as a non-specific rabbit IgG control and had no inhibitory effect. Rabbit anti-BCR Fab fragments had nearly identical inhibitory effects as the whole IgG rabbit anti-BCR. This further suggests that the inhibitory effects of the rabbit anti-BCR are due to specific inhibition of the goat anti-BCR binding and not any factors related to engagement of other receptors such as Fc γ RIIB.

A second strategy to demonstrate the dependence of the antigen presentation enhancement on BCR-mediated uptake of the antigen utilized heat-treated goat anti-BCR. The heat treatment destroyed specific antibody recognition of the BCR but did not destroy the antigenic epitope. Fig. 5A demonstrates the loss of enhancement of antigen presentation by B cells after heat-treating the goat anti-BCR. Fig. 5B demonstrates that the specific epitope was still intact because dendritic cells, which take up antigen by nonspecific mechanisms, still presented the heat-treated anti-BCR as well as untreated antibody. These two strategies confirm that the enhancement we observe in antigen presentation by B cells is BCR-mediated.

3.4. Enhancement in antigen presentation is not due to BCR cross-linking

These experiments sought to confirm that BCR-mediated enhancement of antigen presentation was not mediated merely by crosslinking of BCR, which is a well-known inducer of activation of B cells. We performed antigen presentation experiments in primary B cells where the BCR is cross-linked with anti-BCR but presentation of a different antigen taken up by nonspecific mechanisms was measured. T-cell hybridoma 1ACD5 is specific to an epitope from HIV RT (Jones et al., 2007), and B-cell uptake of RT is not mediated by the BCR. Fig. 6 demonstrates that the addition of goat anti-BCR does not augment presentation of RT by B cells, further supporting our contention that the massive enhancement in BCR-mediated antigen presentation is not significantly enhanced by activation of the B cell, but rather the BCR-mediated uptake mechanism itself.

3.5. Characterization of HLA-DR15 restricted T-hybridoma cell lines and specificity of B-cell antigen presentation

We have generated an HLA-DR15 restricted anti-goat T-cell hybridoma line as well. Using HLA-DR15+ human B-cells and HLA-DR15-restricted T-cell hybridoma 4B6. We have generated results nearly identical with those produced by HLA-DR1 APCs and hybridoma 1C. Fig. 7A illustrates enhanced antigen presentation of goat Ig epitopes by HLA-DR15+ B-cells when using goat anti-BCR compared with goat IgG control. Furthermore, Fig. 7B shows concentration-dependent blocking by rabbit anti-BCR of BCR-mediated antigen presentation in the HLA-DR15 system. This line is also HLA-DR-restricted and not costimulation dependent (data not shown). Generating this line further confirms the utility of this HLADR transgenic mouse-derived T-cell hybridoma system to study human BCR-mediated antigen presentation in human primary B-cells.

3.6. Number of antigen-presenting cells

As a practical consideration, use of the T-cell hybridoma as an *in vitro* tool for analysis of BCR-mediated antigen presentation can eliminate the T-cell tool as a limiting factor in scientific analysis. Fig. 8 illustrates that reducing the number of B-cells reduced the maximal T-cell response, but has a limited effect on antigen presentation relative to antigen concentration. Responses above background were readily seen with as few as 6250 B cells per well.

4. Discussion

The previous development of HLA-restricted transgenic T-cell hybridomas as a tool for measuring human antigen presentation was important to establish these hybridomas as an effective T-cell reagent (Canaday et al., 2003). Subsequently, adaptation of this system to investigate antigen presentation by human B cells became a natural goal to extend the functionality of the hybridoma system. T-cell hybridomas possess several notable advantages to other T-cell reagents in the laboratory. The cell lines can be grown to large numbers using routine cell culture techniques without the need of restimulation and with less risk of losing specificity for its antigen. We believe these to be the first studies demonstrating the applicability of these T-cell hybridomas in measuring BCR-mediated antigen presentation.

Using goat anti-human BCR as an antigen has the practical advantage binding the BCR – regardless of the unique specificity of each BCR. Exploiting this technique required creation of a T-cell hybridoma (1C) specific to one of the non-variable epitopes of goat antibody. We have illustrated that 1C is specific for an epitope on goat Fab, with no detectable cross-reactivity with purified goat Fc or IgG from other species.

The cellular interactions between B cells and T-cell hybridomas adhere to known models of antigen presentation. Interfering with the interaction between the MHC-peptide complex and TCR would be expected to inhibit a T-cell hybridoma response, and we have demonstrated complete blocking of antigen presentation with anti-HLA-DR antibodies. Also typical of T-cell hybridomas, their response is independent of the costimulatory effect provided by B7 (CD80 or CD86) on B cells interacting with CD28 on the T-cell reagent, as addition of antibodies to CD80 and CD86 have no discernable effect on the T-cell hybridoma responses. This relative costimulation independence of T cell hybridoma as a readout of antigen presentation makes this tool a more antigen presentation specific reagent that is a potential advantage for focused mechanistic type studies.

The magnitude of enhancement of BCR-mediated antigen presentation using a T-cell hybridoma system also conforms to established models (Rock et al., 1984; Lanzavecchia, 1985). Antigen presentation by primary B cell is enhanced by a factor of 3 to 5 logs relative to a nonspecific goat IgG control when the antigen enters the B cell through the BCR. Even EBV-infected B-lymphocytes maintained an 1000-fold elevation of antigen presentation using goat anti-human BCR as an antigen, illustrating the potential for studies of human B-cell antigen presentation using all immortalized lines of APC and T-cell hybridoma.

A closer look at the concentration of antigen required to obtain presentation by B cells (Fig. 4A) and dendritic cells (Fig. 4B) suggest that B cells present as efficiently or even more so than dendritic cells when the presentation is BCR-mediated.

The mechanism of BCR-mediated antigen presentation involves the very specific interaction between antigen and the BCR, which on most B cells in the blood includes the IgM molecule. Binding of antigen leads to internalization of the BCR:antigen complex, activation

of the B lymphocyte, and antigen presentation via MHC II. As a result, we set out to demonstrate antigen presentation by human B lymphocytes in a T-cell hybridoma system requires interaction between the antigen and BCR. To this end, we demonstrated that antigen presentation of goat anti-human BCR is inhibited in a dose-dependent manner by the addition of rabbit anti-human BCR, but not by addition of rabbit serum with equivalent amounts of nonspecific rabbit IgG. Saturation of the BCR with the non-antigen rabbit antibody blocked the BCR-mediated uptake of goat anti-BCR that is recognized by the T-cell hybridoma. Furthermore, we were able to demonstrate that heat-treatment of goat anti-human BCR, disrupting its binding affinity for the BCR, effectively silenced antigen presentation of goat epitopes by B cells. To ensure that the relevant epitope was not destroyed with heat treatment, we confirmed that presentation of heat-treated goat anti-BCR is maintained in dendritic cells that do not use BCR-mediated uptake for presentation.

When a unique BCR recognizes its cognate antigen, an essential component of the B cell:helper CD4+ T-cell interaction is the preferential presentation of that specific antigen by the B cell, rather than presentation of other antigens or self proteins that are present. We show in our system that crosslinking the BCR alone does not upregulate all nonspecific uptake mechanisms of antigen in B cells. By crosslinking the BCR with goat anti-human BCR while evaluating the antigen presentation of a second antigen that is taken up by non-specific mechanisms, we confirmed that binding of the BCR does not modulate the antigen presentation of other peptide epitopes, which could otherwise confound the data presented above.

We believe that our system, in which antigen is exposed to the B cell in soluble form, has important implications for the study of B-cell mediated antigen presentation. There is *in vitro* and *in vivo* evidence that the soluble antigen pathway is relevant – particularly in the delivery of small molecules to the B cells in lymph nodes. Roozendaal et al identify a unique pathway for channeling small lymph-borne antigens from the subcapsular sinus directly to the B-cell follicles (Roozendaal et al., 2009). Similarly, Pape et al found that soluble antigens diffuse directly from lymph in the subcapsular sinus to the underlying follicles, where they are acquired by antigen-specific B cells to initiate the humoral response (Pape et al., 2007). However, there is also evidence that macrophages and follicular dendritic cells can capture antigen and share it with B cells. B cells have been shown to recognize the antigen in immune complexes bound to Fc and complement receptors on the surface of both dendritic cells and macrophages (Ahearn et al., 1996; Molina et al., 1996; Barrington et al., 2002). Macrophages have been shown to acquire particulate antigens and have been visualized forming a putative macrophage:B cell synapse to share the specific antigen (Carrasco and Batista, 2007; Junt et al., 2007). The delivery of antigen to B cells in soluble form as well as via cell-to-cell interaction represent two parallel mechanisms. As our methods are currently designed to investigate B-cell recognition of soluble forms of antigen, this is a limitation of the current system. It may be possible however to adapt our T-cell hybridoma system to measure B-cell antigen presentation of antigen delivered by cellular rather than soluble means.

The field of B-cell antigen presentation in humans has had a relative paucity of investigation. We feel that a large part of this is due to limited reagents and techniques to study this cell population in humans. We believe our T-cell hybridomas then are powerful tools that could allow an expansion of studies in human B cells. Areas of consideration could include studying the effects of vaccine adjuvants on presentation function of B cells. Mechanistic studies can be undertaken with EBV-lymphoblasts because they can be genetically manipulated and can be grown to an unlimited supply. Additionally, as our system utilizes the vast majority of B cells in blood, mechanistic studies might now be

feasible on *ex vivo* primary human B cells because smaller volumes of blood can yield more than adequate numbers to study.

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Abbreviations

APC	antigen presenting cell
BCR	B cell receptor
TCR	T-cell receptor
RT	reverse transcriptase
HAT	hypoxanthine/aminopterin/thymidine

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Fig. 1.

Antigen presentation by human dendritic cells. HLA-DR1+ monocyte derived dendritic cells and goat-specific T-cell hybridoma (1C) were incubated with goat IgG, Fab or Fc. In all figures hybridoma responses were determined by the CTLL-2 bioassay and presented as O.D. 550 – O.D. 595. Results represent mean and standard deviation for triplicate wells for all figures. This is representative of two similar experiments.

Fig. 2. Characterization of antigen presentation by human B cells to T-cell hybridomas. HLA-DR1+ B-cells were incubated with goat anti-human BCR and T-cell hybridoma (1C). (A) Addition of mouse anti-human HLA-DR or mouse IgG1 isotype control (10 μg/ml of each). (B) Addition of anti-human CD80 and CD86 (5 μg/ml of each) or mouse IgG1 isotype control (10 μg/ml). Each panel is representative of two similar experiments.

Fig. 3.

Antigen presentation of goat anti-human BCR antibody by human B cells. HLA-DR1+ primary B cells (A) and a EBV-transformed B-cell line (B) were incubated with either goat anti-human BCR or goat IgG and T-cell hybridoma (1C). Panel A is representative of eight similar experiments and panel B four similar experiments.

Fig. 4. Specificity of goat anti-human BCR. HLA-DR1+ B-cells and T-cell hybridoma (1C) were incubated with goat anti-BCR, with addition of rabbit IgG and Fab anti-BCR or rabbit serum as a rabbit IgG control. This is representative of five similar experiments with rabbit IgG and two with rabbit Fab.

Fig. 5.

Antigen presentation of heat-treated goat anti-human BCR. HLA-DR1+ human B-cells (A) and monocyte-derived dendritic cells (B) were incubated with either intact or heat-treated goat anti-human BCR. Heat treatment of goat anti-human BCR was carried out for 20 minutes at 85°C. Panel A is representative of five similar experiments and Panel B three similar experiments.

Fig. 6. Antigen presentation by human B- cells. HLA-DR1+ B-cells were incubated with RT and RT-specific T-cell hybridoma (1ACD5). Goat anti-human BCR was added to demonstrate potential enhancement of RT presentation to 1ACD5. This figure is representative of two similar experiments.

Fig. 7.

Antigen presentation by HLA-DR15+ human B-cells and BCR specificity of antigen presentation. (A) Antigen presentation of goat anti-human BCR or goat IgG by HLA-DR15+ human B- cells. (B) Antigen presentation of goat anti-human BCR with additional of rabbit anti-human BCR as an inhibitor. HLA-DR15+ B-cells and T-cell hybridoma (4B6) were incubated with antigen and an inhibitor or media control. Each panel is representative of two similar experiments.

Fig. 8. Comparison of antigen presentation by varying population numbers of B-cells. Different HLA-DR1+ B-cells per well were incubated with goat anti-human BCR and T-cell hybridoma (1C). This figure is representative of two similar experiments.