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Analysis of costimulatory molecule expression on antigen-specific T and B cells during the induction of adjuvant-induced Th1 and Th2 type responses

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Abstract

Previous studies show that the generation of maximal T cell responses requires B cell antigen presentation and the differential expression of costimulatory molecules by B cells may affect polarization of naïve T cells to Th1 or Th2 phenotypes. We have therefore characterized the expression of activation and costimulatory molecules on antigen-specific T and B cells following immunisation with Alum or Alum/LPS to induce Th2 or Th1 responses in vivo. While antigen-specific B cells show similar levels of activation with respect to MHCII upregulation following Th1 or Th2 induction, they differentially express costimulatory molecules. Although ICOS-B7RP-1 interactions were originally implicated in Th2 generation, surprisingly this receptor–ligand pair was only upregulated on antigen-specific T and B cells following Th1 induction. In conclusion, these studies indicate that during the generation of antigen-specific Th1 or Th2 responses, adjuvants induce differential costimulation in antigen-specific B cells that may subsequently influence T cell polarization.

Keywords: B lymphocytes; Th1/Th2 cells; Costimulation

1. Introduction

Immune responses to T-dependent Ag require stimulation of T cells by paracortical DC with concomitant delivery of costimulatory signals [1]. Without costimulation T cell anergy ensues [2]. Adjuvants can stimulate DC costimulatory molecule expression and bias T cell responses towards a Th1 or Th2 phenotype [3–5], therefore, it is usually assumed that DC costimulation is the major influence on T cell polarization [6]. After stimulation, primed T cells downregulate CCR7 and upregulate CXCR5, directing migration towards the B cell follicle [7–11]. At the follicular boundary, T cells encounter Ag-loaded B cells [12] and provide costimulation and cytokines [13], allowing generation of a B cell response [14–16]. Without T cell help this cannot occur, thus the importance of T cell help for B cell responses is well defined [17,18], however, whether these interactions result in further T cell stimulation is unclear. Ag-specific T cells could receive signals from B cells which allow full T cell differentiation. Indeed, previous studies have suggested that B cells are necessary for maximal CD4 T cell responses [13,19–23]. B cells express several costimulatory molecules that may influence the fate of the T cell, including B7RP-1 (the ligand for ICOS) [24], CD70 [25], OX40 (CD134) [26], OX40L [27], CD30L (CD153) [28]. Interestingly, some of these molecules may be preferentially expressed on B cells and not DC, including CD153 [26] and CD70 [29]. Whether Ag presentation by B cells influences Th1 versus Th2 generation is not known, although it has been suggested that B cell-derived OX40L signals are required to develop Th2 cells [19]. Furthermore, Alum immunization results in accumulation of an IL-4 producing, $GR1^+$ population which enhances B cell activation [30]. It could be hypothesized that this may lead to increased

Abbreviations: Alum, Aluminium hydroxide; PLN, Peripheral lymph nodes

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B cell Ag presentation that could skew responses towards a Th2 phenotype. Thus, additional signals from cognate B cells may facilitate optimal T cell responses and differentiation [31].

In an attempt to identify the molecules involved in the adjuvant-driven differentiation of T cells, we have characterized costimulatory molecules upregulated on Ag-specific B and T cells during Th2 or Th1 induction using Alum or Alum/LPS, respectively [12,32]. We report that the proportion of T cells activated following Th1 immunization was greater than following Th2 immunization, and these cells remained activated for longer periods than Th2 cells. During both responses Ag-specific B cells were activated to similar levels with respect to MHCII expression, although they differentially upregulated costimulatory molecules. Surprisingly, despite being described as a Th2 costimulator, ICOS and its ligand, B7RP-1, are preferentially upregulated on Ag-specific T and B cells, respectively, during Th1 induction.

2. Materials and methods

2.1. Animals

IgHb (H-$2^{d/d}$, IgM^b), and $Ly5.1^+$ (H-$2^{b/b}$, IgM^b) mice were bred in house (Central Research Facilities, University of Glasgow). DO11.10 mice with OVA-specific T cells [33] were used as Tg T cell donors and MD4 mice with Hen Egg Lysozyme (HEL)-specific B cells [34] were used as Tg B cell donors for transfer into 6–12-week-old IgHb recipients. Alternatively, 6–12-week-old $Ly5.1^+$ recipients received OVA-specific OTII Tg T cells and MD4 Tg B cells. All animals were specified pathogen free and were maintained under standard conditions in accordance with local and Home Office regulations.

2.2. Preparation of cell suspensions for adoptive transfer

Cells were prepared as described previously [12,35,36]. Briefly, cell suspensions were made from peripheral lymph nodes (PLN), mesenteric lymph nodes and spleens from MD4, OTII or DO 11.10 mice. Suspensions were washed and the percentage of MD4 B cells, OTII or DO11.10 T cells was determined by flow cytometry (see below). Unirradiated, age-matched IgHb BALB/c or Ly5.1 C57BL6 recipients received 1–6 $\times 10^6$ tg T and 1–6 $\times 10^6$ tg B cells intravenously.

2.3. Ags and Ag administration

Chicken OVA (Fraction V) was obtained from Sigma (Worthington Biochemical Corporation, USA) and Hen Egg Lysozyme from Biozyme (Gwent, UK). Conjugated OVA-HEL was prepared as described previously [35]. The absence of contaminating endotoxin was confirmed through LAL assay, or lack of effects on dendritic cells in vitro. Animals received 130 μg OVA-HEL in 1% Alum (Brenntag Biosector, Frederikssund, Denmark) with or without 1 μg LPS (*Salmonella abortus equi*)(*Sigma*) or 1 μg IL-12 (PeproTech, London, UK) subcutaneously (s.c.).

2.4. Flow cytometry

PLN were harvested 1–5 days after immunization. Cell suspensions were prepared as above. Cells were stained for flow cytometry as described previously [12,35,36]. Briefly, cells were initially incubated with anti-CD16/32. $CD4^+$ DO11.10 or OTII Tg T cells were detected with biotinylated-KJ1.26 [37] or biotinylayed-anti-Ly5.1 (BD Pharmingen, Oxford, UK), respectively, followed by fluorescein isothiocyanate (FITC)-streptavidin (SA) (Vector, Peterborough, UK) and anti-CD4-phycoerythrin (PE) (BD Pharmingen). Anti-B220-PE (BD Pharmingen) and biotinylated-anti-IgM^a (BD Pharmingen)/SA-FITC or anti-Ly5.1-bio/SA-FITC were used to identify MD4 B cells. Two colour analysis was performed on 20,000 events. For three colour staining, Tg T cells were stained with anti-CD4-Peridinin Chlorophyll Protein (PerCP) (BD Pharmingen), KJ1.26-bio or biotinylated-anti-Ly5.1 followed by SA-FITC, and anti-CD69-PE or anti-ICOS-PE or an isotype control of irrelevant specificity (all BD Pharmingen). For CD25 staining anti-CD25-FITC (BD Pharmingen) was used and SA-PE was substituted for SA-FITC. Tg B cells were stained with anti-B220-PerCP, biotinylated-anti-Ly5.1/SA-PE, and anti-MHCII-FITC, anti-CD80-FITC, anti-CD40-FITC or an isotype control of irrelevant specificity (all BD Pharmingen). For B7RP-1 staining, cells were incubated with anti-B220-PerCP, biotinylated-anti-Ly5.1/SA-FITC, then biotinylated-anti-B7RP-l (BD Pharmingen) and SA-PE. Analysis was performed on 1000–5000 tg cells per sample.

2.5. Antibody ELISAs

To detect anti-HEL IgM^a in serum, Immulon 2 plates (Corning-Costar, New York, USA) were coated with HEL (20 μg/ml) at 4 °C overnight, then washed three times with PB S Tween (0.05%, Sigma) before being blocked with PBS-FCS (10%, v/v) for 1 h at 37 °C. Plates were washed and incubated with diluted serum samples (1/100) for 3 h at 37 °C before washing. Biotinylated-anti-IgM^a (2 μg/ml) was added for 1 h at 37 °C. Plates were washed and incubated with Extravidin-URP (l/1000)(Sigma) for 1 h at 37 °C.

Plates were washed again and TMB Microwell Peroxidase Substrate (Kirkegaard and Perry Laboratories, Gaithersburg, MD, USA) was added. Reactions were stopped with 10% H_2O_2 (Fisher Scientific International, Leicester, UK). Absorbances were read on a plate reader at 450 nm.

2.6. Statistics

Results are expressed as mean ± S.E.M. To test significance, Student's unpaired *t*-tests were performed. A *p*-value of <0.05 was regarded as significant.