Human B cells promote T-cell plasticity to optimize antibody response by inducing coexpression of T_H1/T_{FH} signatures

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Background: B cells mediate humoral immunity against pathogens but also direct $CD4^+$ T-cell responses. Recent plasticity studies in mice have challenged the concept of strict fate commitment during $CD4^+$ T-cell differentiation into distinct subsets.

Objective: We sought to elucidate the contribution of human antigen-primed B cells in $CD4^+$ T-cell responses that support humoral immunity.

Methods: $CD4^+$ T-cell differentiation by primary human B cells was investigated in *in vitro* cocultures by using tetanus toxoid and *Salmonella* species as antigen models. T-cell differentiation was assessed by using intracellular cytokines and subset-specific transcription factors and markers. IgM and IgG formation was analyzed by means of ELISA.

Results: Human B cells, but not dendritic cells, induce prominent and stable coexpression of T_H1 and follicular helper T (T_{FH}) cell characteristics during priming and on antigen recall. T_H1/T_{FH} cells coexpress the T_H1 and T_{FH} effector cytokines IFN-γ and IL-21 and the T_{FH} marker CXCR5, demonstrating that the coexpressed T_H1 and T_{FH} subset–specifying transcription factors T-box transcription factor (T-bet) and B cell lymphoma 6 are both functionally active. B cell–derived IL-6 and IL-12 controlled respective expression of IL-21 and IFN-γ, with IL-21 being key for humoral immunity.

Conclusion: Human B cells exploit $CD4^+$ T-cell plasticity to create flexibility in the effector T-cell response. Induction of a T-cell subset coexpressing IL-21 and IFN-γ might combine IL-21–mediated T-cell aid for antibody production while maintaining T_H1 cytokine expression to support other cellular immune defenses. (J Allergy Clin Immunol 2015;135:1053-60.)

Key words: *B cell, T-cell plasticity, IL-21, IFN-γ, coexpression, T-bet, Bcl-6, antibody response, crosstalk*

Abbreviations used

Bcl-6:	B cell lymphoma 6
DC:	Dendritic cell
DP:	Double positive
ICOS:	Inducible costimulator
LN:	Lymph node
PD-1:	Programmed cell death 1
PE:	Phycoerythrin
SP:	Single positive
STAT:	Signal transducer and activator of transcription
T-bet:	T-box transcription factor
T_{FH}:	Follicular T helper
TT:	Tetanus toxoid

On infection, the generation of pathogen-specific IgG antibodies requires differentiation of antigen-specific B cells. This process often requires help by defined $CD4^+$ T_H cell subsets, of which follicular T helper (T_{FH}) cells seem most dedicated to the task.[1-5] T_{FH} cells are characterized by the transcription factor B cell lymphoma 6 (Bcl-6), various surface markers (CXCR5, programmed cell death 1 [PD-1], and inducible costimulator [ICOS]) and the hallmark cytokine IL-21,[6,7] which supports B-cell proliferation and IgM, IgG, and IgA formation.[8]

The origin of the T_{FH} cell and its relation to other T_H subsets remains a matter of intense ongoing research. Data in mice show T_{FH} cell fate commitment,[9,10] but they can also show plasticity by coexpressing different subset-specific transcription factors and displaying shared $T_{FH}/T_H1/T_H2$ phenotypes.[11-16] In human subjects small IFN-γ^+/IL-21^+ double-positive $CD4^+$ memory cells exist in healthy individuals[17] and in patients with inflammatory bowel disease,[18] but their origin and function remain unclear.

Classically, activated dendritic cells (DCs) regulate T_H cell differentiation and thus define the quality of T-cell help to B cells. Recently, however, the clinical effects of B-cell depletion with anti-CD20 mAbs showed that B cells might also regulate $CD4^+$ T-cell differentiation.[19,20] Indeed, the T_{FH} cell differentiation pathway in mice depends in part on B cells.[21-24]

To address the contribution of antigen-primed B cells to T_H cell differentiation, we investigated B-cell/T-cell interactions by using several model antigens. We show that human antigen-primed B cells induce a stable T_H1/T_{FH} intermediate that strongly promotes antibody secretion. These data show that human B cells can exploit $CD4^+$ T-cell plasticity to generate coexpression of effector T-cell molecules, which might enable an optimal coordination of different immune responses against specific pathogens.

METHODS

Antibodies

Anti–IL-21 (PeproTech, Rocky Hill, NJ), anti–IL-4 (eBioscience, San Diego, Calif), anti–IFN-γ (U-CyTech Biosciences, Utrecht, The Netherlands),

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anti–IL-12p35 (B-T21; Gen-Probe, Bedford, Mass), and anti–IL-6 (Sanquin, Amsterdam, The Netherlands) were used as blocking antibodies. Labeled anti-human mAbs were used in flow cytometry: IFN-γ, IL-4, Bcl-6, dendritic cell-specific intercellular adhesion molecule-3- (ICAM-3) grabbing non-integrin (DC-SIGN), CD4 (BD Biosciences, San Jose, Calif), IL-21, T-box transcription factor (T-bet; eBioscience), CXCR5, CXCL13, and Bcl-6 (R&D Systems, Minneapolis, Minn).

Lymphocyte isolation and generation of monocyte-derived DCs

Human PBMCs were isolated from buffy coats from healthy donors (Sanquin Blood Supply) by using Ficoll-Hypaque (Axis-Shield PoC AS, Dundee, Scotland). Donors were evaluated as healthy based on medical check-up and questionnaires and provided written informed consent. The study was approved by the Medical Ethics Committee of Sanquin. B and T cells were purified with anti-CD19 and anti-CD4 Dynabeads (Invitrogen, Carlsbad, Calif). Untouched naive ($CD4^+CD45RO^-$) and memory $CD4^+$ T cells ($CD4^+CD45RA^-$) were purified (>98%) through CD45RO–phycoerythrin (PE) or CD45RA-PE and anti-PE beads (MACS; Miltenyi Biotec, Bergisch Gladbach, Germany). Monocyte-derived DCs were generated, as previously described.[25] Matched PBMCs and lymph nodes (LNs) derived from surgical residual material were collected from kidney transplant recipients on informed consent. Cell suspensions were obtained by grinding LN pieces through a flow-through chamber.

Antigen-primed B-cell/T-cell cocultures

B cells or immature DCs were infected with exponentially growing *Salmonella typhimurium* SL1344 and green fluorescent protein–expressing *Salmonella* species, as previously described.[26] When high numbers of antigen-presenting B cells were required to facilitate B-cell differentiation analyses, uptake of *Salmonella* species was enhanced through coating of *Salmonella* species with anti-IgM/anti-LPS tetrameric antibody complexes because IgM B cells form the majority of *Salmonella* species–specific B cells and their targeting allows analysis of IgM to IgG class-switching.[26] *Salmonella* species–infected cells (1×10^5) were cultured with 5×10^4 $CD4^+$ T cells.[26] IL-21 (50 ng/mL, Invitrogen), IFN-γ (Immukine; 1000 IU/mL), and IL-4 (50 ng/mL; Janssen Biochemica, Beerse, Belgium) were added when described. For experiments with tetanus toxoid (TT), B cells and T cells were cultured in 1:1 ratio (5×10^4 cells each) in the presence of 0.1 μg/m: TT (Statens Serum Institut, Copenhagen, Denmark).

Flow cytometry and flow imaging

Proliferation of carboxyfluorescein succinimidyl ester (Invitrogen)–labeled B and T cells was measured after 6 days. 4′-6-Diamidino-2-phenylindole dihydrochloride (Sigma-Aldrich, St Louis, Mo) was used to analyze living cells. Cytokine production was measured by using intracellular staining at day 11 of coculture and after 5 hours of restimulation with 0.1 μg/mL phorbol 12-myristate 13-acetate, 1 μg/mL ionomycin, and 10 μg/mL brefeldin A (Sigma-Aldrich), as previously described.[27]

Monocyte-derived DCs were incubated with anti-IgM–coated, green fluorescent protein–expressing *Salmonella* species for 1 hour in medium without antibiotics and were cultured in the presence of gentamicin after washing. Cells were analyzed using ImagestreamX (Amnis, Seattle, Wash) with IDEAS software (Amnis). Single cells were gated on *Salmonella*-positivity, and only cells and *Salmonella* species detected in the focal plane were selected for further analyses.

ELISA

IgM and IgG levels in supernatants were measured, as previously described.[26] In short, plates were coated with polyclonal anti-IgM (SH15, Sanquin) or anti-IgG (MH16, Sanquin), and for detection, anti-IgM–horseradish peroxidase (MH15, Sanquin) or anti-IgG–horseradish peroxidase (MH16.1, Sanquin) was used.

Real-time semiquantitative RT-PCR

RT-PCR has been described elsewhere.[26] Primers were developed to span exon-intron junctions and validated (see Table E1 in this article's Online Repository at www.jacionline.org). Gene expression was measured in duplicate by using SYBR green and the ABI PRISM 7000 Sequence Detection System (Applied Biosystems, Foster City, Calif). Results were normalized to *18S* rRNA and expressed relative to expression in $CD4^+$ T cells stimulated with unprimed B cells.

Statistical analysis

The paired Student *t* test and GraphPad Prism software (version 5.01; GraphPad Software, La Jolla, Calif) were used. Data are shown as means + SEMs, with *P* values shown in figure legends.

RESULTS

Human B cells induce T-cell polarization toward IFN-γ and IL-21

The role of primary human B cells in $CD4^+$ T_H cell polarization was investigated by using different antigen-specific *in vitro* models. We previously showed that internalization of *Salmonella* species by specific B cells induces presentation of *Salmonella*-antigens to $CD4^+$ T cells and $CD4^+$ T cell–mediated antibody production.[26,27] *Salmonella*-primed B cells become activated, proliferate, and induce a $CD4^+$ T-cell response, whereas unprimed B cells hardly induce CD4 T-cell proliferation (see Fig E1 in this article's Online Repository at www.jacionline.org), yielding very low absolute numbers of cytokine-producing T cells. In the absence of B cells, *Salmonella* species did not activate T cells (see Fig E2 in this article's Online Repository at www.jacionline.org). As in mice,[28] antigen-primed human B cells significantly expanded IFN-γ^+ T cells (Fig 1, *A*). Costaining with anti–IL-21 mAb showed significant IL-21 induction, yielding an IL-21^+ single-positive (SP) but also a prominent IFN-γ^+/IL-21^+ double-positive (DP) population (13.5%; Fig 1, *B*). Only a few IL-4^+/IL-21^+ DP cells were formed. IL-17 induction was very low, and IL-10 was not detected (data not shown). Thus *Salmonella*-positive B cells support both T_H1 and T_{FH} cell polarization.

The use of TT as an alternative model antigen also led to proliferation of TT-primed B cells (see Fig E3, *A*, in this article's Online Repository at www.jacionline.org) and $CD4^+$ T cells (see Fig E3, *B* and *C*). Again, a prominent IFN-γ^+/IL-21^+ DP cell population was induced (Fig 1, *C* and *D*), demonstrating that various antigen-primed B cells induce IFN-γ and IL-21 coexpression in $CD4^+$ T cells. *Salmonella* species was chosen to investigate the IFN-γ^+/IL-21^+ DP cell population in more detail because it induced the strongest response.

IL-21 induction is a B cell–specific process and favors antibody secretion

Salmonella-primed monocyte-derived immature DCs (see Fig E4 in this article's Online Repository at www.jacionline.org) and B cells were compared for their T-cell polarization capacities. In contrast to antigen-primed B cells, antigen-primed DCs did not induce IL-21 (Fig 2 and see Fig E5 in this article's Online Repository at www.jacionline.org), demonstrating that IL-21 expression on *in vitro* stimulation of CD4 T cells is mediated by B cells rather than DCs. In line with earlier observations that CD4 T-cell activation in the B-cell/T-cell cocultures is