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Germinal Center Helper T Cells Are Dual Functional Regulatory Cells with Suppressive Activity to Conventional CD4+ T Cells

Ekaterina Marinova, Shuhua Han, and Biao Zheng

Germinal center (GC) reaction drives a series of important events, including clonal expansion and selection of Ag-specific B cells, somatic hypermutation of Ig genes, affinity maturation of Ab responses, and generation of B cell memory (1–3). The cellular components in GCs include follicular dendritic cells (FDCs), which define the locus of GC formation by retaining unprocessed Ags, Ag-specific helper T and B cells, which migrate to and proliferate within the FDC reticulum, and tingible body macrophages, which are responsible for clearing up the apoptotic cells in GC. GC helper T cells are a requisite component of the GC response; in the absence of T cell help, GCs are not formed or are functionally defective (4–7). Ag-mediated BCR cross-linking promotes B cell localization at the boundary between the T cell zone and B cell follicles (8–11), which is the location of the initial encounters between Ag-bearing B cells and Ag-specific T cells. After activation, some B cells migrate to the medullary cords of lymph nodes or splenic red pulps and differentiate into Ab-producing plasma cells. Other activated B cells move to the follicles and undergo massive clonal expansion to form GCs (1–3). At the same time, Ag-specific Th cells must migrate into the follicular area to support B cell response in the GCs (12–16). One historical paradox is why the GC microenvironment only allows B cells but not T cells to undergo massive proliferation, although a significant number of CD4+ T cells do present within the GC.

The lack of immune activities mediated by conventional CD4+ T cells in the GC may be either due to their intrinsic inability to migrate to the GC site or they are constantly purged from the locale. It has been shown that homing of B cells to the GC area is dependent on the expression of CXCR5 by B cells and production of its ligand CXCL13 by follicular stromal cells and FDCs (17–20). The localization of T cells in the GC environment is also largely mediated by CXCL13. After activation, T cells up-regulate CXCR5 and down-regulate CCR7, acquiring responsiveness to CXCL13 while simultaneously becoming less responsive to the T cell zone chemokines such as CCL19 and CCL21 (21, 22). It was suggested that CXCR5 expression may define T cells with B cell helper function and those CXCR5+ T cells localized in the follicles were termed follicular B helper T cells (Tfh) (23, 24). However, available data have shown that CXCR5+ T cells are functionally heterogeneous and CXCR5 expression is not restricted to GC helper T cells but is widely expressed on activated CD4+ T cells (17, 18, 21, 25, 26). Thus, the lack of significant activity of conventional CD4+ T cells in the GC is unlikely due to their intrinsic inability to respond to CXCL13 produced in the lymphoid follicles.

In the current study, we report that human tonsil CD4+CD57+ T cells, which are the major helper T cells found exclusively in the GCs (25–28), strongly inhibit the proliferation and function of conventional CD4+ T cells through Fas ligand (L), TGF-β, and IL-10. By contrast, CD4+CD57+ GC T cells effectively promote GC B cell activation, survival, and Ab production. Thus, CD4+CD57+ T cells are a novel population of bifunctional regulatory cells within the GC that support B cell responses but suppress T cell responses.

Materials and Methods

Abs and reagents

For isolation of various lymphocyte subsets, the following anti-human mAbs were used: biotinylated anti-CD3, CD4, CD8, CD16, Mac-1, and CD57 Abs were obtained from BD Pharmingen. Streptavidin (SA), anti-CD45RO, and anti-mIgG1 microbeads were purchased from Miltenyi Biotec. For surface and intracellular staining, anti-CD45RO-PE or allophycocyanin, anti-CD4-PE, PerCP or allophycocyanin, anti-CD57-FTC, anti-CD19-FTC, anti-CD25-PE, anti-CCR7-PE, biotinylated anti-FasL, Annexin V-PE or FITC, anti-IL-10-PE, anti-TGF-β-biotin, and SA-PerCP were all obtained from BD Pharmingen; anti-glucocorticoid-induced


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2 Abbreviations used in this paper: GC, germinal center; FDC, follicular dendritic cell; L, ligand; SA, streptavidin; mlgG1, mouse IgG1; Treg, regulatory T cell; FoxP3, forkhead box P3; GITR, glucocorticoid-induced TNF-like receptor; Tr1, type 1 T regulatory; Th3, type 3 Th.

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TNF-like receptor (GITR)-FITC, anti-CTLA-4-PE, anti-CD134-PE, mlgG1-FITC, mlgG1-PE, mlgG2a-PE, and purified anti-human CD38 were purchased from eBioscience; and anti-mouse IgG1-biotin and anti-CD40-biotin were purchased from Caltag Laboratories.

**Purification of CD4+ T cell subsets from tonsils**

Human tonsil cells were prepared from individual tonsils removed during routine tonsillectomy. Tonsils were minced in cold PBS supplemented with 2% calf serum, and mononuclear cells were purified by centrifugation through an ionic density gradient medium (Lymphoprep Medium; Nycomed Pharma) as described previously (26, 28). Erythrocytes were lysed and then the cell preparations were depleted of B, NK, and CD8 cells by MACS separation following the manufacturer’s instructions (Miltenyi Biotech). Resulting CD4+ T cells were incubated with biotinylated anti-CD57 Ab and SA microbeads and then GC T cells were separated by positive selection. Positively selected cells were passed over an additional column to obtain increased purity. CD4+ CD57+ T cells were further incubated with anti-CD45RO microbeads to isolate memory/effector (CD4+ CD45RO+ CD57+) and naive (CD4+ CD45RO- CD57-) T cell subsets. CD25+ or CD57+ regulatory T cells (Treg) were purified by FACS using MoFlow (DakoCytomation). The purity of each isolated T cell subset was routinely 90–99%. To purify CD19+ B cell subsets, tonsil cells were first depleted of CD4, CD8, and NK cells by MACS. GC B cells were further separated from non-GC B cells by positive selection after incubation with purified anti-human CD38 and mlgG1 microbeads. The purity of each isolated B cell subset was 95–99%.

**Cell culture and proliferation**

CD4+ CD57+ T cells (responders) were cultured in U-bottom 96-well microtiter plates (Costar; Corning) either alone (2 × 10⁶ cells/well) or with CD4+ CD57+ GC T cells of various ratios (1:1, 1:1/2, 1:1/4, etc.) in RPMI 1640 medium (Invitrogen Life Technologies) with 10% FCS and 1% l-glutamine supplemented with antibiotics. Equal numbers of purified CD19+ B cells were added as APCs. Cells were stimulated with plate-bound anti-CD3 mAb (1.0 μg/ml; BD Pharmingen) and 0.5 μg/ml anti-CD28 (eBioscience) and cultivated for 4 days at 37°C with 5% CO₂. To measure cellular proliferation, isolated CD4+ CD57+ responder T cells were labeled with 5 μM CFSE (Molecular Probes) for 15 min at 37°C, washed three times, and cultured as described above. Transwell experiments were conducted in 24-well plates with responders CD4+ T cells in the bottom and GC T cells on transwell membranes (Costar; Corning). Cells were stimulated with plate-bound anti-CD3 and soluble anti-CD28 Abs. For blocking or neutralizing experiments, cells were cultured as described above, but in the presence or absence of neutralizing mAbs as follows: anti-TGF-β1, 2, β2 (R&D Systems) in the concentration of 5 and 10 μg/ml, anti-IL-10 (R&D Systems) in the concentration of 5 and 10 μg/ml, anti-IL-12 (BD Pharmingen) in the concentration of 2.5 and 5 μg/ml. Functional grade mouse IgG1, NA/LE (BD Pharmingen) and mouse IgG2b (eBioscience) were used as isotype control Abs in the same concentrations. For T-BC cell cocultures, purified GC B cells (10⁵/well) were cultured with either purified GC T or non-GC T cells (10⁵/well) in the presence of 5 μg/ml soluble anti-CD3 and 2 μg/ml anti-CD28 mAbs. After 96 h, cell surface expression of CD19, CD80, CD86, and CD40 was analyzed by flow cytometry. The number of live GC B cells per well was calculated by multiplying the number of total live cells by the percentage of CD19+ cells.

**Flow cytometry and intracellular staining**

T cells were stimulated with plate-bound anti-CD3 and soluble anti-CD28 Abs for 3 days. Cells were then stimulated with 50 ng/ml PMA and 500 ng/ml. Anti-granzyme A-PE, anti-granzyme B-PE, anti-CD86, and CD40 was analyzed by flow cytometry. The purity of each isolated T cell subset was 95–99%.

**Real-time PCR**

Cells were sorted to >96% purity. Total RNA was purified using TRIzol (Invitrogen Life Technologies) and reverse transcribed into cDNA with oligo(dT) using the SuperScript kit (Invitrogen Life Technologies). The cDNA was amplified in triplicate in real-time PCR using a TaqMan PCR kit (Applied Biosystems) with primers for GAPDH, PTEN, forkhead box P3 (Foxp3), GITR, IL-10, and TGF-β purchased as Assay on Demand (Applied Biosystems). mRNA levels were normalized relative to GAPDH mRNA expression.

**Measurement for Ab concentrations**

Equal numbers (5 × 10⁴/well) of purified B cells and T cell subsets were cultured for 10 days and supernatant Ab levels were determined by ELISA. Samples were incubated on ELISA plates coated with anti-mouse Ig followed by HRP-conjugated goat anti-mouse IgM or IgG. HRP activity was visualized using a tetramethylbenzidine peroxidase substrate kit (Bio-Rad) and ODs were determined at 450 nm.
CD4^+ CD57^+ GC T cells support GC B cell response but suppress conventional CD4^+ T cells

To determine whether the CD4^+ CD57^+ GC T cell population exerts differential effects on B cell and T cell responses, we first examined the efficacy of CD4^+ CD57^+ T cells in promoting GC B cell survival, expression of molecules important in T-B collaboration, and Ab production. When purified GC B cells were cocultured with CD4^+ CD57^+ GC T cells or conventional CD4^+ CD57^- non-GC T cells, we found that GC B cells survived much better when they were cocultured with CD4^+ CD57^+ GC T cells than with CD4^+ CD57^- non-GC T cells (Fig. 1A). Importantly, GC B cells expressed significantly higher levels of CD40, CD80, and CD86 when they were cocultured with CD4^+ CD57^+ GC T cells than with conventional CD4^+ CD57^- T cells (Fig. 1B). In addition, when the effectiveness in supporting Ab production was compared between different CD4^+ T cell subsets, we found that CD4^+ CD57^- GC T cells were more effective than conventional CD4^+ CD57^- naive, memory, or effector T cells (Fig. 1C), consistent with previous work showing that GC T cells promote tonsil B cells to secrete Abs in vitro (25, 29, 30).

Next, we investigated whether GC T cells exert a regulatory effect on reactivity of conventional CD4^+ CD57^- T cells. Suppression of cellular proliferation was determined by in vitro coculture of CD4^+ CD57^- GC T cells and CFSE-labeled conventional...
CD4\textsuperscript{+}CD57\textsuperscript{+} T cells as responders. Our results showed that >80% of cellular proliferation of CD4\textsuperscript{+}CD57\textsuperscript{+} responder T cells was inhibited by CD4\textsuperscript{+}CD57\textsuperscript{+} GC T cells (Fig. 2A). The degree of suppression was dependent on the ratio of CD4\textsuperscript{+}CD57\textsuperscript{+} GC T cells:CD4\textsuperscript{+}CD57\textsuperscript{+} responder T cells in cultures, showing a clear dose-dependent suppression curve (Fig. 2B). Consistently, IL-2 production by responding CD4\textsuperscript{+}CD57\textsuperscript{+} T cells was also suppressed by CD4\textsuperscript{+}CD57\textsuperscript{+} GC T cells (Fig. 2C). A similar result was received by intracellular staining of IL-2 in the responder cells (data not shown). Thus, we have established that CD4\textsuperscript{+}CD57\textsuperscript{+} GC T cells possess dual regulatory functions in that, on one end, they provide critical help signals to promote B cell responses, on the other hand, they also suppress conventional CD4\textsuperscript{+} T cell responses.
To determine whether CD4\(^+\)/CD57\(^+\) GC T cells differentially regulate Th1- or Th2-mediated immune responses, we have examined the effect of CD4\(^+\)/CD57\(^+\) GC T cells on Th1 and Th2 cytokine production by conventional CD4\(^+\)/CD57\(^-\) T cells. Our data demonstrated that, under neutral condition (responder T cells were stimulated with anti-CD3 and anti-CD28 only), CD4\(^+\)/CD57\(^+\) GC T cells inhibited both Th1 (IFN-\(\gamma\)) and Th2 (IL-4) cytokine production by conventional CD4\(^+\) T cells (Fig. 3A). Under Th1-polarizing conditions (responder T cells were activated in the presence of IL-12, IL-18, and anti-IL-4), CD4\(^+\)/CD57\(^+\) GC T cells suppressed IFN-\(\gamma\) production but were unable to inhibit cellular proliferation of conventional CD4\(^+\) T cells (Fig. 3B). However, when responder T cells were activated under Th2-polarizing conditions (addition of IL-4 and anti-IFN-\(\gamma\)), CD4\(^+\)/CD57\(^+\) GC T cells were unable to suppress either cellular proliferation or IL-4 production (Fig. 3C). Thus, CD4\(^+\)/CD57\(^+\) GC T cells have a predominant regulatory effect on the Th1-mediated immune response in that they are able to inhibit Th1 cytokine production even under strong Th1-polarizing conditions.

Suppression mediated by CD4\(^+\)/CD57\(^+\) GC T cells is partially dependent on cognate cell-cell contact via CD95-CD95L interaction

We then investigated the mechanisms of suppression mediated by CD4\(^+\)/CD57\(^+\) GC T cells. Using a Transwell culture system, CD4\(^+\)/CD57\(^+\) GC T cells were separated from conventional CD4\(^+\) T cell responders stimulated with plate-bound anti-CD3 and soluble anti-CD28 mAb. We found that separation of GC T cells from their target conventional CD4\(^+\) T cells by Transwell membranes partially restored proliferation of conventional CD4\(^+\)/CD57\(^+\) T cells (Fig. 4, A and B). These findings suggest that suppression of conventional CD4\(^+\)/CD57\(^+\) T cells by CD4\(^+\)/CD57\(^+\) GC T cells was partly dependent on cell-cell contact.

To identify the molecule(s) that is involved in the cell-cell contact-mediated suppression, we investigated the unique expression pattern of surface molecules by CD4\(^+\)/CD57\(^+\) GC T cells. We found that, although CD95 was up-regulated on all Ag-experienced (CD45RO\(^+\)) T cell subsets (data not shown), CD4\(^+\)/CD57\(^+\) GC T cells, accounting for >95% among CD95L-expressing CD4\(^+\) T cells (Fig. 4, C and D). To determine whether CD95L mediates suppression by CD4\(^+\)/CD57\(^+\) GC T cells, we blocked CD95-CD95L interaction by using a neutralizing Ab specific for CD95L. Our data show that proliferation of CD4\(^+\)/CD57\(^+\) T cells was indeed partially restored by the addition of anti-CD95L Ab (Fig. 4, E and F), confirming that cognate contact-dependent suppression by GC T cells was delivered through CD95-CD95L interaction.
We have further investigated the roles of other candidate molecules that may be involved in cell-cell contact-dependent suppression by CD4⁺ CD57⁺ GC T cells, including granzyme A and granzyme B. However, results from flow cytometric analysis of intracellular proteins and real-time RT-PCR analysis of mRNA expression indicated that these molecules were not expressed by CD4⁺ CD57⁺ GC T cells ex vivo and were not significantly up-regulated after activation by TCR cross-linking in vitro (data not shown). Therefore, cytolytic activity mediated by these molecules unlikely plays a significant role in the cell-cell contact-mediated suppression by CD4⁺ CD57⁺ GC T cells.

TGF-β and IL-10 are required for the full-scale suppression mediated by CD4⁺ CD57⁺ GC T cells

Because a cell-cell contact-dependent mechanism alone could not account for the potent suppression by CD4⁺ CD57⁺ GC T cells, we investigated the involvement of soluble factor-dependent mechanisms in GC T cell-mediated suppression. We have determined the cytokine profile of CD4⁺ CD57⁺ GC T cells by ELISA and intracellular cytokine staining. Our data showed that, in addition to low levels of IL-2, IL-4, and IFN-γ (data not shown), CD4⁺ CD57⁺ GC T cells produced significant levels of IL-10 and TGF-β. Among all of the IL-10- or TGF-β-producing CD4⁺ T cells, ~80% were CD4⁺ CD57⁺ GC T cells, whereas conventional activated (CD45RO⁺) CD4⁺ CD57⁻ T cells only constituted ~20% of IL-10- or TGF-β-producing cells (Fig. 5A), although the population size of conventional CD4⁺ CD57⁻ T cells was much bigger (~5-fold) than that of CD4⁺ CD57⁺ GC T cells (26).

To determine the role of IL-10 and TGF-β in GC T cell-mediated suppression, we investigated whether neutralization of IL-10 or TGF-β during coculturing of CD4⁺ CD57⁺ GC T cells and conventional CD4⁺ CD57⁻ responder T cells would reverse the suppression. Our data showed that neutralization of either TGF-β or IL-10 by neutralizing Ab (Fig. 5B) partially abolished the suppression by GC T cells, indicating that both IL-10 and TGF-β are required for the full-scale suppression.

To determine whether CD95L, IL-10, and TGF-β together are responsible for all of the mechanistic components that mediate suppression by CD4⁺ CD57⁺ GC T cells, we tested whether neutralization of all three molecules by an Ab mixture would completely reverse the suppression by CD4⁺ CD57⁺ GC T cells. Our findings (Fig. 5C) showed that suppression of CD4⁺ CD57⁻ responder T cells by CD4⁺ CD57⁺ GC T cells was indeed completely abolished by the combination of all three neutralizing Abs against CD95L, IL-10, and TGF-β, suggesting that these three molecules contribute to most of, if not all, the mechanisms of suppression mediated by CD4⁺ CD57⁺ GC T cells.

CD4⁺ CD57⁺ GC T cells represent a novel population of Treg that express CTLA-4 and GITR but not Foxp3

To further characterize CD4⁺ CD57⁺ GC T cells, we examined their expression of a panel of surface molecules and compared it to that of conventional CD4⁺ T cell subsets. Flow cytometry analysis of freshly isolated CD4⁺ T cell populations showed that both CD25 and the ICOS molecule were similarly up-regulated between CD4⁺ CD57⁺ GC T cells and conventional CD4⁺ CD57⁻ memory/effectector T cells (Fig. 6, A and B), whereas CTLA-4 (CTLA-associated protein 4) up-regulation on CD4⁺ CD57⁺ GC T cells was more pronounced than on memory/effectector T cells (Fig. 6C). CCR7 expression was down-regulated on CD45RO⁺ (GC and memory/effectector) T cells compared with naïve T cells (Fig. 6D), indicating that both GC T cells and memory T cells may have decreased responsiveness to the T-zone chemokines. Our data also demonstrate that GC T cells and memory/effectector T cells express similar levels of CD40L, CD62L, CD69, and HLA-DR (data not shown). Thus, except that GC T cells are CD57⁺ CD95L⁺ and express a higher level of CTLA-4, GC T cells and conventional CD4⁺ effector T cells are similar in their phenotypic profiles.

To investigate whether CD4⁺ CD57⁺ GC T cells share characteristic features with other known T cells with regulatory functions, including the naturally arising Treg, type 1 T regulatory (Tr1), and type 3 Th (Th3) cells, we analyzed the expression of genes that is related to Treg function and compared it between Treg cells and CD4⁺ CD57⁺ GC T cells. Using real-time RT-PCR analysis, we found that CTLA-4 expression was comparable between GC T cells and Treg cells (Fig. 7A). Another important molecule for Treg is GITR, which is constitutively expressed on

FIGURE 7. GC T cells exhibit a unique phenotype distinct from other Treg subsets. GC (CD4⁺ CD45RO⁻ CD57⁺) T cells (Tgc) from tonsil, Treg (CD4⁺ CD25high) from peripheral blood, or naive (CD4⁺ CD45RO⁺ CD57⁻ CD25⁻) T cells (T naive) from tonsil were purified by FACS and subjected to real-time RT-PCR. mRNA levels were normalized relative to GAPDH mRNA expression. Similar results were obtained from three independent experiments.
Treg and inducibly expressed on conventional CD4+CD25− effector T cells (31, 32). Our data show that GITR expression on GC T cells was similar to that on Treg cells (Fig. 7B). However, CD4+CD57+ GC T cells do not express the unique molecular marker of Treg cells, transcription factor Foxp3 (Fig. 7C). Therefore, CD4+CD57+ GC T cells are a novel Treg population that is phenotypically and mechanistically distinct from other T cell subsets with immune regulatory functions including Treg, Tr1, and Th3 cells.

Discussion
This study reveals that CD4+CD57+ GC T cells are bifunctional regulatory cells in that they not only provide effective help function for B cell response, but also display suppressive function toward conventional CD4+ T cells, revealing a novel mechanism that secures the GC microenvironment for effective Ag-specific B cell activation and differentiation.

The GC environment is exclusively reserved for optimal B cell response and lack of significant immune activities of conventional cells. One apparent mechanism responsible for the absence of immune responses mediated by conventional CD4+ effector T cells such as the Th1 response is that they are unable to respond to the same signals that mediate the recruitment and retention of GC B cells and helper T cells. It has been established that the recruitment of both B cells and helper T cells to the GC area is largely determined by their responsiveness to the chemokine CXCL13 produced in the lymphoid follicles (17–21). Thus, lack of immune activities mediated by conventional T cells may result from their inability to express the receptor for CXCL13 and CXCR5. However, existing evidence has shown that CXCR5+ T cells are quite heterogeneous (17, 18, 21, 25, 26), in that they include activated T cell subsets of various functions. Therefore, CXCR5 expression may be more appropriately considered as a marker for most activated T cells rather than a marker for T cells with B cell help function in the follicular area.

Because T cells can up-regulate CXCR5 expression after activation, most effector T cells have the potential to gain access to the GC environment. However, only GC T cells with B cell helper function are retained in the GC area. These facts suggest that there is a mechanism that actively purges the local environment of other T cells that may cause interference and, thus, ensures an efficient Ag-specific B cell response in the GC. This hypothesis led us to explore whether a component of the GC response can actively suppress immune responses mediated by conventional CD4+ effector T cells. CD4+CD57+ T cells are the major helper T cells found exclusively in the GCs of human tonsils (25–28). Therefore, we investigated whether CD4+CD57+ GC T cells display differential regulatory effects on the B cell response and immune activities of conventional CD4+ T cells. Remarkably, our findings show that the same CD4+CD57+ GC T cell population not only provides the most efficient help signals for GC B cell response but also potently suppresses other CD4+ T cells, demonstrating for the first time that the same T cell subset can exert differential effects on different immune cells.

Our current studies show that CD4+CD57+ GC T cells are a novel type of regulatory cells that is phenotypically and mechanistically distinct from other known T cells with regulatory function. It is largely agreed that the primary mechanism of suppression mediated by CD4+CD25+Foxp3+ Treg involves direct cell-cell contact between suppressor and effector T cells (33, 34). Experimental evidence indicates that the ligation of CD80 and CD86 on effector T cells by CTLA-4 on Treg may explain the contact requirement between effector and suppressor T cells (35, 36). Previous work has also shown that contact-dependent mechanisms of suppression by Treg may involve cytolytic activity mediated by granzyme A, granzyme B, and perforin, but do not involve CD95-CD95L binding (37, 38). In contrast, CD4+Tr1 cells, which differentiate in the periphery from naive precursors in the presence of IL-10, regulate T cell responses through their ability to produce IL-10 and TGF-β (39, 40). Although existing evidence has suggested that CD4+CD25+Foxp3+ Treg may also suppress T cell responses through the production of IL-10 and/or TGF-β (41, 42), the involvement of cytokine-dependent suppression by CD4+CD25+Foxp3+ Treg is controversial (33, 43–46). Another type of T cells with regulatory function is Th3, which are usually generated during oral tolerance induction by low doses of Ags and exert their suppressive effect through TGF-β (47). Our data demonstrate that CD4+CD57+ GC T cells do not belong to any of those T cell subsets with regulatory function in that they not only have a unique phenotypic profile, but also use a unique combination of suppressive mechanisms. Phenotypically, although CD4+CD57+ GC T cells up-regulate CD25, CTLA-4, and GITR, they are fundamentally different from Treg because they do not express Foxp3 (Fig. 6). Mechanistically, although suppression by CD4+CD57+ GC T cells is partially dependent on cognate cell-cell contact, this requirement for direct contact is clearly different from that of Treg, in that the contact-dependent suppression by GC T cells is mediated through CD95-CD95L interaction and independent of granzyme A, granzyme B, or perforin. Furthermore, although CD4+CD57+ GC T cells mediate suppression partially through production of IL-10 and TGF-β, they are clearly distinct from Tr1 and Th3 cells.

In summary, CD4+CD57+ GC T cells are a novel T cell subset with true “regulatory” function, which has been previously termed as “hermaphrocytes” (48) because they possess both positive and negative regulatory activities. On one hand, CD4+CD45RO+CD57+ GC T cells are helpers in that they promote the Ag-specific B cell response through cell-cell contact via CD40-CD40L and soluble factors such as IL-4 and IL-10 (49, 50). On the other hand, as we have shown in the current study, CD4+CD57+ GC T cells are suppressors in that they inhibit conventional effector T cells by cell-cell contact and by cytokines including IL-10 and TGF-β.

Disclosures
The authors have no financial conflict of interest.

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