ICOS Deficiency Is Associated with a Severe Reduction of CXCR5 +CD4 Germinal Center Th Cells

Lukas Bossaller, Jan Burger, Ruth Draeger, Bodo Grimbacher, Rolf Knoth, Alessandro Plebani, Anne Durandy, Ulrich Baumann, Michael Schlesier, Andrew A. Welcher, Hans Hartmut Peter and Klaus Warnatz

J Immunol 2006; 177:4927-4932; doi: 10.4049/jimmunol.177.7.4927
http://www.jimmunol.org/content/177/7/4927

References
This article cites 31 articles, 13 of which you can access for free at: http://www.jimmunol.org/content/177/7/4927.full#ref-list-1

Subscription
Information about subscribing to The Journal of Immunology is online at: http://jimmunol.org/subscription

Permissions
Submit copyright permission requests at: http://www.aai.org/About/Publications/JI/copyright.html

Email Alerts
Receive free email-alerts when new articles cite this article. Sign up at: http://jimmunol.org/alerts

The Journal of Immunology is published twice each month by The American Association of Immunologists, Inc., 1451 Rockville Pike, Suite 650, Rockville, MD 20852
Copyright © 2006 by The American Association of Immunologists All rights reserved.
Print ISSN: 0022-1767 Online ISSN: 1550-6606.
ICOS Deficiency Is Associated with a Severe Reduction of CXCR5\(^+\)CD4 Germinal Center Th Cells\(^1\)

Lukas Bossaller,* Jan Burger,† Ruth Draeger,* Bodo Grimbacher,* Rolf Knoth,‡ Alessandro Plebani,§ Anne Durandy,‖ Ulrich Baumann,‖ Michael Schlesier,* Andrew A. Welch,§ Hans Hartmut Peter,* and Klaus Warnatz\(^2\*)

ICOS is expressed on activated T cells and particularly on CXCR5\(^+\) follicular Th cells in germinal centers (GC). Its deletion leads to a profound deficiency in memory B cell formation and switched Ab response in humans. Here, we show that in ICOS-deficient patients the generation of GCs is severely disturbed, and the numbers of circulating CXCR5\(^+\)CD45RO\(^+\) memory CD4 T cells are significantly reduced, indicating an essential role of ICOS in the differentiation of CXCR5\(^+\)CD4 T cells. The GC-specific CD57\(^+\)CXCR5\(^+\) subpopulation is virtually absent. In ICOS\(^{−/−}\) mice, the decrease of circulating CXCR5\(^+\)CD4 T cells reflects the reduction of CXCR5\(^+\) follicular Th cells in lymph nodes and spleen. Therefore, in concurrence with the absence of CXCR5\(^+\) T cells in the blood of CD40L-deficient patients, these data support the hypothesis that circulating CD57\(^+\)CXCR5\(^+\) T cells are GC derived and thus may serve as a surrogate marker for the presence of functional GCs in humans. The Journal of Immunology, 2006, 177: 4927–4932.

A hallmark of T-dependent immune responses is the formation of germinal centers (GC)\(^3\) within B cell follicles of secondary lymphoid organs. This process involves directed migration of T and B cells to enable colocalization and costimulation between resident follicular dendritic, T, and B cells (1). The fine tuning of this migration is achieved by the expression of chemokines by resident cells and their respective receptors by the migrating cell.

Through the expression of the chemokine receptor CCR7, T cells are usually attracted by dendritic cells expressing high levels of the CC chemokines secondary lymphoid tissue chemokine and EBV-induced molecule 1 ligand chemokine into the T cell zone (2–4). Activated CD4 T cells up-regulate the expression of another chemokine receptor, CXCR5 (5). The CXCR5-specific ligand CXCL-13 (synonym, BLC or BCA-1) is expressed mainly by reticular and endothelial cells in B cell follicles (6). The coexpression of CXCR5 and CCR7 positions newly activated T cells at the border of T and B cell zone. Further down-regulation of CCR7 responsiveness attracts CXCR5\(^{high}\)-expressing CD4 T cells into B cell follicles (7). The CXCR5\(^{high}\)CD4 T cells provide an excellent cognate help for Ab-producing B cells in vitro and therefore were termed follicular Th cells (T\(\\text{FH}\)) (8–10). Since then, several follicular CD4 T cell populations have been distinguished in human tonsils (11, 12). CD57\(^+\)CXCR5\(^+\) T cells preferentially localize to the light zone of GC and produce elevated levels of IL-10 and IL-21 upon activation. They are more efficient in inducing Ab production by B cells than CD57\(^−\)CXCR5\(^−\) T cells, which are localized in a rim between mantle zone and the light zone of the GC.

Within tonsils, T\(\\text{FH}\) cells express several activation markers including high levels of a costimulatory molecule called ICOS (9). ICOS is a member of the CD28 family and is induced on activated CD4 T cells and subsequently in the GC formation and Ab responses (13). ICOS-deficient mice (14–16) show poor T-dependent Ab responses and a defective GC formation. Recently, we described the first patients with a homozygous deletion of ICOS (17) presenting with the clinical phenotype of common variable immunodeficiency. On the basis of the coexpression of CXCR5 and ICOS on T\(\\text{FH}\) cells in established GCs as well as the defective GC formation and Ab responses in ICOS deficiency, we asked whether ICOS deficiency affects circulating CXCR5\(^+\)CD4 T cells. Our results demonstrate a profound diminution of circulating CXCR5\(^+\)CD45RO\(^+\) memory CD4 T cells in human ICOS deficiency, suggesting that ICOS is involved in the generation of CXCR5\(^+\)CD4 T cells and subsequently in the GC formation and humoral immune responses. Furthermore, the findings suggest that circulating CXCR5\(^+\) memory CD4 T cells derive from follicular precursors.

Materials and Methods

Patients

Nine previously described patients (17, 18) with the molecular diagnosis of ICOS deficiency were enrolled into ethical board-approved protocols at the University of Freiburg. Three patients with the molecular diagnosis of CD40L deficiency were seen at the university clinic in Hannover (Germany), Brescia (Italy), or Paris (France). Informed written consent was obtained from each patient before participation.
Animals
ICOS-deficient animals (14) were backcrossed to the BALB/c background using marker-assisted accelerated backcrossing (Charles River Laboratories). Marker analysis indicated that the backcrossing was >99.37% complete. Eight- to twelve-month-old ICOS-deficient female BALB/c mice and age- and sex-matched controls were used for these experiments and were held under specific pathogen-free conditions in the mouse house at the Institute of Virology (Freiburg, Germany).

mAbs
Abs to human proteins were purchased from the following sources: anti-CD57 (NK-1), anti-OX40 PE (ACT35), anti-CD154 PE (TRAP1), anti-CD19 PC7 (J4.119), and anti-CD69 FITC (FN50) from BD Pharmingen; anti-CXCR5 PE (51S05.111) and R&D Systems; anti-CD4-APC (1B3B2.2) and anti-CD45RO-FITC (UCHL1) from Immunotech; anti-CD3-PC7 (UCHT1) from Beckman Coulter; and hamster anti-ICOS-biotin (15F9) from E Bioscience.

Abs to murine proteins were purchased from the following sources: anti-CXCR5-biotin (2G8), anti-CXCR5-PE (2G8), anti-OX40-ligand-biotin, anti-IgM-FITC, anti-OX40-biotin (OX-86), anti-CD154-biotin (MR1), anti-TCR-β-chain FITC (H57-597), anti-CD4-PerCP-Cy5.5 (RM4-5) and anti-CD5/B220-PE (RA3-6B2) as well as isotype control PE-labeled mouse IgG1k Abs from BD Pharmingen for data for isotype controls not shown; anti-CD45/B220-PerCP (RA3-6B2) from BD Biosciences; PNA-biotin from Vector Laboratories; anti-CD3-Alexa 647 (clone 553141), and biotinylated Abs were detected with streptavidin-PE-labeled mouse IgG1k Abs from BD Pharmingen (data for isotype controls not shown); anti-CD45/B220-PE (RA3-6B2) from BD Biosciences; PNA-biotin from Vector Laboratories; anti-CD3-Alexa 647 (clone 553141), and biotinylated Abs were detected with streptavidin-PE-labeled mouse IgG1k Abs from BD Pharmingen.

Isolation, depletion, and in vitro activation of human CXCR5+ CD4 T cells
EDTA blood (50–80 ml) was drawn from nine ICOS-deficient patients and controls. Mononuclear cells (PBMC) were isolated by Ficoll-Hypaque density gradient centrifugation (Biochrom) and cultured as previously described (17). CD4 T cells were positively selected by Dynabeads (CD4+ isolation kit; Dynal) and further purified by incubation (30 min, 4°C) with anti-CD14, anti-CD16 and anti-CD19 mAbs (Coulter-Immunotech; purity >96%). CXCR5+ T cells were separated from positively selected CD4 T cells by cell sorting on a MoFlo high speed cell sorter (DakoCytomation). Purified CD4 T cells (105 cells/ml) were stimulated for 18 h in 24-well cell culture dishes (Greiner) precoated with 2.5 μg/ml goat F(ab)2’, anti-mouse IgG (Jackson Immunoresearch Laboratories) and 200 ng/ml monoclonal anti-CD3 Ab (clone Hit3a; BD Pharmingen), 200 ng/ml monoclonal anti-CD28 Ab (clone CLB-CD28/1; Sanquin Reagents), or 4 μg/ml anti-ICOS (clone F44; see Ref. 13). After exclusion of dead cells by propidium iodide staining, cells were analyzed for CXCR5, CD40L, OX40 and ICOS expression. For the analysis of cell proliferation, purified and CXCR5+ T cells were incubated for 10 min at 37°C in PBS with 1 mM carboxyfluorescein diacetate (Molecular Probes) and then stimulated in vitro for 3–4 days and stained as described above.

Immunization protocol
For primary T-dependent responses, mice were immunized i.p. with 50 μg of hen egg lysozyme (HEL; Serva Biochemicals) in CFA (Invitrogen Life Technologies) and sacrificed on day 10 postimmunization. We collected blood from the tail vein and separated serum from blood lymphocytes using Lympholyte-M (Cedarlane Laboratories) for gradient centrifugation. Blood from the tail vein and separated serum from blood lymphocytes were incubated for 10 min at 37°C in PBS with 1 mM carboxyfluorescein diacetate (Molecular Probes) and then stimulated in vitro for 3–4 days and stained as described above.

ELISA for the detection of anti-HEL Abs
Plates coated with 10 μg/ml HEL were incubated for 2 h at room temperature with serial dilutions of mouse pre- and postvaccination sera. Bound HEL-specific IgG or IgM Abs were detected with goat anti-mouse IgG-AP, specific for Fcγ, or with μ-chain-specific goat anti-mouse IgM-AP Abs (all Jackson Immunoresearch). Substrate reaction was performed with p-nitrophenylphosphate (Sigma-Aldrich) in diethamolanine buffer.

In vitro Transwell migration assays
Migration assays were performed with 5 x 104 PBMCs in 100 μl of RPMI-0.5% BSA in the upper well of Transwell Costar Plates (65 mm diameter and 5.0 μl pore size) (Corning) and 640 μl of RPMI-0.5% BSA plus 2000 ng of human BLC (R&D Systems) in the lower well. After 2 h at 37°C, 5% CO2 cells in the lower well were collected, stained, and counted by flow cytometry (19).

Histology of lymph node and spleen
For immunohistochemical analysis, cryosections of murine spleens and lymph nodes were incubated with biotinylated anti-CXCR5 Ab overnight at 4°C. After three washes in PBS, the sections were incubated in staining buffer with FITC (B220 and PNA) and PE (CD4 and Thy1)-conjugated Abs and streptavidin-Cy5 (Amersham; 1/80) for detection of biotinylated CXCR5. After a washing in PBS, slides were incubated for 5 min with 4,6-diamidino-2-phenylindole (Serva), washed again, mounted in Fluoromount G (Southern Biotechnologies), and analyzed by microscopy. Quantification of CD4 T cells in 10–15 different follicular regions was performed by counting CD4 T cells/100 μm2. Digitized images were analyzed with the Open Lab Software (Improvement).

Immunohistochemistry of paraffin sections of human lymph nodes was performed according to standard protocols. CD3 expression was detected using the Vectastain ABC Kit and anti-CD3 Ab (clone F7.2.38; DakoCytomation).

Statistical analysis
Statistical analysis was performed using an unpaired and paired Student t test, when appropriate.

Results
Peripheral blood of human ICOS-deficient patients is severely depleted of CXCR5+ CD4 T cells which coincides with a disturbed GC formation in secondary lymphoid tissue
Circulating CD45RO+CD4 T cells of healthy individuals contain 5–15% of CXCR5+ T cells. These cells had been suggested to derive from GC Tfh cells (8), but unlike their tonsillar counterparts, these cells are resting and express CCR7 on the surface. In ICOS-deficient patients, we found a severe reduction of circulating CXCR5+ CD4 T cells as well as an almost complete absence of CD57+CXCR5+CD4 T cells (Fig. 1, A and B). This was not due to a general reduction of CD45RO+ memory CD4 T cells (Fig. 1, A and B). The low CXCR5 expression in FACS analysis was corroborated by a markedly reduced migration of freshly isolated ICOS-deficient CD4 T cells to the CXCR5 ligand CXCL13 compared with T cells of healthy donors (data not shown). This migration was not restored to normal after T cell stimulation with anti-CD3/CD28 (see also below). The expression of the other homoeostatic chemokine receptors CCR7 (Fig. 1B), and CXCR4 on CD4 T cells as well as the expression of CXCR5 on B cells was not altered (data not shown), suggesting a specific dysregulation of the development or maintenance of CXCR5+ T cells in the absence of ICOS. This drastic reduction of CXCR5+ T cells was unusual among other hypogammaglobulinemic patients with normal ICOS expression (50 common variable immunodeficiency patients; data not shown). Interestingly, as in ICOS−/− mice (14), GC formation in one examined lymph node of an ICOS-deficient patient was severely disturbed (Fig. 1C).

ICOS costimulation increases CXCR5 up-regulation of activated CD4 T cells but does not by itself prevent consecutive CXCR5 down-regulation
To investigate the mechanism leading to a reduced CXCR5 expression on CD4 T cells from ICOS-deficient patients we asked whether ICOS costimulation is directly involved in CXCR5 up-regulation. Isolated normal CD4 T cells were stimulated in vitro with anti-CD3 or anti-CD3/anti-ICOS. Maximal CXCR5 expression was observed at 16–20 h of stimulation (data not shown). In healthy donors, costimulation through ICOS consistently increased the expression level of CXCR5 (anti-CD3: mean intensity of fluorescence (MIF), 93 ± 28.7; anti-CD3/anti-ICOS: MIF 121 ± 40.6; p < 0.002) (Fig. 2, A and B).
ICOS-deficient T cells showed poor up-regulation of CXCR5 after activation with anti-CD3 (or anti-CD3/anti-ICOS), which partially improved after costimulation with anti-CD3/anti-CD28 (Fig. 2A). Due to the absence of preexisting circulating CXCR5+ T cells in ICOS-deficient patients, a direct comparison between ICOS-deficient patients and healthy donors was complicated. Therefore, we depleted T cells of healthy donors of CXCR5+ cells. These depleted CD4 T cells of healthy donors showed only a weak up-regulation of CXCR5, undistinguishable from total CD4 T cells of ICOS-deficient patients (Fig. 2A).

After the initial up-regulation, in vitro activated CXCR5+ T cells decreased the surface CXCR5 receptor expression after day 3 (8, 11). In our examination, CXCR5 expression began to decline as early as after 16 h of stimulation, whereas ICOS expression was further up-regulated at this point (Fig. 2C). We speculated that ICOS may therefore play a role in the maintenance of high CXCR5 surface expression on CXCR5+ T cells keeping CD4 T cells within B cell follicles. To test this hypothesis, isolated CD4 T cells were stimulated with anti-CD3/anti-CD28 for 16 h, before anti-ICOS was added. In this assay, the down-regulation of CXCR5 was not altered by the mere addition of ICOS costimulation (data not shown), not excluding a role of ICOS in the context of a more complex stimulatory milieu of forming GCs.

FIGURE 1. ICOS deficiency causes a severe reduction of circulating CXCR5+ CD4 memory T cells and disturbed GC formation in humans. A. Dot plots show a representative flow cytometric analysis of CD3+CD4 T cells from a healthy donor (HD) and an ICOS-deficient patient (ICOS) for CXCR5 vs CD45RO or CD57 expression. Numbers indicate percentage of CD3+CD4 T cells of the respective quadrant. B. Dot graphs demonstrate percentages of CD3+CD4 T cells from healthy donors and ICOS-deficient patients. C. Paraffin section of an inguinal lymph node of an ICOS-deficient patient (ICOS pat.) and a control patient (HD) had been stained for T cells (anti-CD3 in red). T cells zones (T), B cell zones (B), and one GC are indicated.

FIGURE 2. ICOS costimulation up-regulates CXCR5 expression on CD4 T cells but does not generate CXCR5highCD4 memory T cells in vitro. A. Purified CD4 T cells of ICOS-deficient patients (ICOS), controls (HD), and sorted CXCR5+ CD4 T cells of a healthy donor (HD CXCR5 depleted) were stimulated in vitro for 18 h with anti-CD3, anti-CD3/anti-ICOS, or anti-CD3/anti-CD28. Representative dot plots of propidium iodide+CD3+ T lymphocytes are shown. Percentages of CXCR5+ CD45RO+ T cells and MIF of CXCR5 expression on cells in the upper right quadrant are given. B, Intraindividual comparison of CXCR5 expression (MIF) of CD4 T cells from healthy donors after stimulation with anti-CD3 vs anti-CD3/anti-ICOS at 18 h. C, CXCR5 receptor expression of healthy donor-derived CD4 T cells declines during proliferation after anti-CD3/anti-CD28 stimulation, whereas ICOS expression increases. Carboxyfluorescein diacetate (CFDA)-labeled CD4-purified T cells were stimulated for 3–4 days. MIF levels of CXCR5+ and ICOS+ cells are given. The representative results of three similar experiments are shown.
In summary, ICOS costimulation is involved in the initial up-regulation of CXCR5 by freshly activated T cells, but the lack of the CXCR5high T cell subpopulation in ICOS deficiency is a result of a distinct function of ICOS in vivo.

Blood, lymph node, and spleen of naive ICOS-deficient mice contain reduced numbers of CXCR5+ CD4 T cells

For further analysis, we extended to in vivo studies in ICOS−/− mice and analyzed CXCR5 expression of murine CD4 T cells in blood, lymph node, and spleen. In naive, aged (8- to 12-mo-old) BALB/c mice, 2–9% of peripheral CD4 T cells from blood and spleen express CXCR5, in lymph nodes the percentage amounts to 4–7%. CXCR5+ CD4 T cells of ICOS−/− mice were significantly reduced in all three compartments. Only 0.8–3.9% of circulating T cells (p < 0.004), 0.9–2.8% of splenic T cells (p < 0.02), and 1.2–5% of the T cells in the lymph node (p < 0.002) express CXCR5 (Fig. 3), but unlike that of humans the blood of ICOS−/− mice contains CXCR5high-expressing CD4 T cells. CXCR5 expression has previously been suggested to depend on the engagement of OX40 on activated T cells (20). Therefore, we investigated if ICOS deficiency is associated with reduced OX40 expression. Our studies did not reveal any difference in the expression of OX40 in the spleen of ICOS−/− and wild-type (wt) mice before (1.9% vs 2%) and after the immunization with HEL-CFA (2.4% vs 2.6%) (data not shown).

Lack of GC in ICOS deficiency is associated with decreased numbers CXCR5+ CD4 T cells in B cell follicles of immunized mice

To elucidate the role of ICOS in the development of CXCR5+ CD4 T cells during Ag-specific immune responses, we immunized four ICOS−/− and four control mice with the T cell-dependent Ag HEL i.p. Our data confirm the failure to establish full GCs in the absence of ICOS (14–16). Lymph nodes from ICOS−/− mice contained only 0.3–0.4% PNA+ GC B cells as opposed to 6–9% in lymph nodes of wt animals (Fig. 4A). IgM as well as IgG Ab responses to HEL were reduced (Fig. 4B). After immunization with HEL, the number of CXCR5+ CD4 T cells increased in the spleen of wt animals (14–23%) at day 10. A similar increase of CXCR5+ Ag specific T cells had been described after immunization with pigeon cytochrome c (10). In ICOS−/− mice, the respective increase (6–7%) in the spleen was lower, but detectable (Fig. 4, C and D). The immunohistological analysis of lymph nodes demonstrated, besides the previously described absence of GCs, a severe reduction of CD4 T cells localizing to B cell follicles of ICOS−/− mice compared with wt mice after immunization (Fig. 4, E and F).

Circulating CXCR5+ CD4 T cells originate from GC reaction

The origin of circulating CXCR5+ memory T cells and their relation to follicular Tfh cells of secondary lymphoid tissue have been discussed controversially (2). The association of severely reduced numbers of circulating CXCR5+ T cells with the impaired GC formation in human ICOS deficiency as well as the correlation of reduced Tfh cells in lymph nodes and spleen of ICOS−/− mice with the reduction of circulating CXCR5+ T cells in the blood strongly support the idea that circulating CXCR5+ T cells derive from a follicular precursor population. We tested our hypothesis by analyzing the blood of three patients with CD40L deficiency, which is known to abrogate GC formation. Like ICOS-deficient patients, all examined patients showed a severe reduction of circulating CXCR5+ T cells (Fig. 5). This was endorsed by the absence of CXCR5+ T cells in the cord blood of immunologically naive newborns.

Discussion

The development and maintenance of GCs during the immune response require cognate T cell help (21). Beside CD40L (22, 23) and CD28 (24), ICOS signals play a crucial role in this process (14–16). However, the exact pathogenic mechanism disrupting the organization of GCs during the advancing immune response in ICOS-deficient mice and humans has not been uncovered thus far. Here we demonstrate that the interaction of ICOS and ICOS-L is essential for the differentiation and/or survival of human as well as murine CXCR5+CD4 T cells, and we suggest that this may be a prerequisite for GC development and/or its maintenance.

Besides potential ICOS-ICOS-L interaction in inflammatory peripheral tissues, ICOS is probably first engaged during the cognate interaction of activated CD4 T and B cells at the T-B border in secondary lymphoid tissues (25). There, ICOS costimulation directly contributes to the up-regulation of CXCR5 on activated Ag-specific ICOS+ T cells, counterbalancing the constitutive expression of CCR7 and thus facilitating the subsequent migration into the adjacent B cell follicle (7). In line with this supposition is our observation of a reduced number of CD4 T cells in B cell follicles of immunized ICOS−/− mice compared with B cell follicles of control littermates. The enhanced activation induced up-regulation of CXCR5 may contribute to the role of ICOS in the T-dependent immune response but is not unique to ICOS and therefore certainly not sufficient to explain the essential role of ICOS in the emergence of the GC-specific CD57+CXCR5highCD4 T cell population (26). The second encounter of ICOS takes place in the light zone of GCs where ICOS is highly expressed on CD57+CXCR5+ Tfh cells (9, 11). At this point, the superinduction of IL-10 (27) and possibly IL-21 (28) by the costimulation via ICOS are thought to be essential for further differentiation of memory and effector B cells (26). Therefore, the reduction of CXCR5+ Tfh in B cell follicles of ICOS deficiency may contribute to reduced local cytokine concentrations and subsequently to the impaired class
switch and memory B cell development. At the same time, ICOS may be an essential survival signal for CD57/CXCR5 TFH cells linking ICOS costimulation, CD57/CXCR5 TFH cells, and GC formation very closely together. Interestingly, in the human lymph node of one ICOS-deficient patient, we could detect some rudimentary bcl-6-positive GCs (29), arguing for a role of ICOS in the completion and/or maintenance rather than initiation of GCs. Recently, Akiba et al. (30) also described the role of ICOS in the maintenance of murine CXCR5 follicular Th cell. Using several mouse strains, the authors demonstrated the common role of ICOS in all tested strains. At the same time, they found interstrain variations regarding the function of OX40. This finding is in line with the lack of OX40 expression on TFH cells of BALB/c mice observed in this study. Interestingly, the requirements for GC development seem to vary between lymph node and spleen in some of the examined strains (30).

Human ICOS deficiency results in an even greater loss of CXCR5 CD4 T cells than the murine counterpart. ICOS deficiency depletes circulating CD57/CXCR5 T cells and significantly reduces CD57/CXCR5 CD4 T cells which also exhibit a CD45RO memory phenotype. The relationship between these two populations remains to be clarified in view of the identification of several CXCR5 populations (12, 31) in the follicles of human tonsils.

As a result of our study in human ICOS deficiency and the murine data of Akiba et al. (30), some new light has been shed on the relationship between circulating CXCR5 CD4 T cells and their activated counterparts in tonsils. Thus, we propose that circulating CD57/CXCR5 CD4 T cells are GC derived, because in ICOS deficiency their reduction parallels the reduction of

FIGURE 4. Rudimentary GC formation and poor primary immune response correlate with low number of circulating CXCR5 CD4 T cells and CXCR5 CD4 TSH cells in B cell follicles of ICOS–/– mice after vaccination with T-dependent Ag. A, Dot plots show PNA IgM–/– GC B cells after gating on B220+ B cells in lymph nodes (LN) of ICOS–/– vs control mice 10 days (d) post-i.p. vaccination with HEL/CFA. B, Primary HEL-specific IgM and IgG responses of ICOS–/– vs control mice 10 days post-i.p. vaccination with HEL/CFA. C, Dot plots show representative flow cytometric analysis of CXCR5 CD4 T cells in the spleen of BALB/c (wt) vs ICOS–/– mice. Numbers indicate percentage of TCRαβ−CD4 T cells in the right upper quadrant. D, Dot graphs demonstrate percentages of CXCR5 CD4 T cells within the population of TCRαβ −CD4 T cells in the spleen from BALB/c (wt) and ICOS–/– mice 10 days post-i.p. vaccination with HEL/CFA. E, Cryosections were prepared from periaortic LN of immunized BALB/c (wt) and ICOS mice. A and B, The T cell zone is marked by anti-Thy1-PE (red) and the B cell zone by anti-B220 FITC staining (green). C and D, Enlargements of indicated areas of A and B and demonstrate the lack of PNA GC cells (GC, anti-PNA FITC; green) and the reduced number of CD4 T cells (anti-CD4-PE; red) in B cell follicles (B, anti-CXCR5-streptavidin-Cy5 (blue) staining) of ICOS–/– mice. F, Comparison of CD4 T cell numbers in B cell follicles of lymph nodes from naive vs immunized (im) ICOS–/– and BALB/c mice. Bars indicate mean and SD.

FIGURE 5. Absence of circulating CXCR5 memory CD4 T cells and CXCR5 CD57+ T cells in CD40L (40L)-deficient patients and cord blood (CB). A, Dot plots show a representative flow cytometric analysis of CD3 CD4 T cells from a CD40L-deficient patient and from cord blood for CXCR5 vs CD45RO or CD57 expression. Numbers indicate percentage of CD3 CD4 T cells of the respective quadrant. B, Dot graphs demonstrate percentages of CD3 CD4 T cells from healthy donors (HD), three CD40L-deficient patients, and five different cord bloods.
CXCR5+CD4 T cells in secondary lymphoid tissue of mice and is associated with the absence of GCs in mice and men. A tight correlation of circulating CXCR5+ T cells with the presence of GCs was further supported by two observations: 1) in three human CD40L-deficient patients known to lack GCs, circulating CXCR5+CD4 T cells were largely absent; 2) cord blood of immunologically naive newborns lacks circulating CXCR5+CD4 T cells. Interestingly, unlike in CD40L deficiency and in cord blood, the CD45RO+ T cell population was not severely diminished in ICOS deficiency, suggesting a more specific block of the CXCR5+ T cell subset in the absence of ICOS.

Our findings shed new light on the key role of ICOS in the development of the GC-associated Tfh cells and provide an explanation for the severe impairment of GC reaction, class switch recombination, and memory B cell development in human ICOS deficiency. Thus, human ICOS deficiency and CD40L deficiency are the first genetic defects with a significant reduction of circulating CXCR5+ memory T cells. We therefore suggest diagnostically using circulating CD57+CXCR5+CD4 T cells as a surrogate marker for the presence of functional GCs in humans. Screening of patients with Ab deficiencies for circulating CXCR5+ memory T cells will help to identify a subset of patients with defective GCs including ICOS deficiency.

Acknowledgments

We thank H. P. Pircher, H. R. Rodewald, and H. Eibel for their help and support with the immunizations and analysis of mice; M. Orlowksa-Volk for discussion; and H. Eibel and M. Lipp for critically reading the manuscript. We thank U. Salzer and J. Horn for their care of the patients; C. Blum, S. Gutenberger, M. Follo, and K. Geiger for technical support; and H. Eibel and M. Lipp for critically reading the manuscript. We thank U. Salzer and J. Horn for their care of the patients; C. Blum, S. Gutenberger, M. Follo, and K. Geiger for technical support; and H. Eibel and M. Lipp for critically reading the manuscript.

Disclosures

The authors have no financial conflict of interest.

References