J Clin Exp Hematop
Vol. 55, No. 2, Nov. 2015

Original Article

Follicular Dendritic Cell Meshwork in Angioimmunoblastic T-Cell Lymphoma Is Characterized by Accumulation of CXCL13$^+$ Cells

Haruo Ohtani,[1,2)] Takuya Komeno,[3)] Yoshiko Agatsuma,[4)]
Motohiro Kobayashi,[5,6)] Masayuki Noguchi,[7)] and Naoya Nakamura[8)]

Angioimmunoblastic T-cell lymphoma (AITL) is considered to originate from follicular helper T (T_{FH}) cells. Currently, neoplastic cells in AITL are considered to express CXCL13 as a tumor marker. However, the identification of CXCL13$^+$ cells remains unclear in terms of whether they are neoplastic cells (or T_{FH} cells) or follicular dendritic cells (FDCs) in both AITL and normal germinal centers. Therefore, the exact identification of CXCL13$^+$ cells was performed using 33 cases of AITL and normal germinal centers. Single-labeling immunohistochemistry and double-labeling immunofluorescent microscopy first confirmed that CXCL13 was expressed mainly in FDCs in the normal germinal centers. In 28 of 33 AITL cases, CXCL13 was expressed mainly in FDCs as a meshwork pattern, which was associated with CXCL13$^+$ neoplastic cells. In the other five cases, CXCL13 was expressed mainly in neoplastic cells, which were densely distributed in and around the FDC meshwork. These findings indicate the abundance of CXCL13$^+$ cells in the FDC meshwork irrespective of the cell type. Triple-labeling immunofluorescent microscopy showed that the CXCL13$^+$ FDC meshwork in AITL harbored both neoplastic cells and B cells. CXCR5, the cognate receptor of CXCL13, was expressed in neoplastic cells in AITL. The present study suggests that neoplastic cells in AITL preserve a certain level of T_{FH}-cell function since neoplastic cells and B cells are closely enmeshed in the CXCL13$^+$ cell-rich FDC meshwork in a similar way as in normal germinal centers. 〔*J Clin Exp Hematop 55(2) :* 61-69*, 2015*〕

Keywords: angioimmunoblastic T-cell lymphoma, CXCL13, follicular helper T cells, follicular dendritic cells, CXCR5

INTRODUCTION

Angioimmunoblastic T-cell lymphoma (AITL) is a unique peripheral T-cell lymphoma (PTCL) composed of the proliferation of not only neoplastic T cells but also various reactive host cells including high endothelial venules (HEVs), B cells often with a blast form, plasma cells, and follicular dendritic cells (FDCs) that form a disorganized meshwork structure (hereinafter called the FDC meshwork in AITL).[1-3] Of these, the proliferation of both FDCs and HEVs distinguishes AITL from other PTCLs.[4] PTCLs that lack either the proliferation of FDCs or that of HEVs are designated AITL-like PTCLs in the present paper. AITL is currently considered to originate from follicular helper T (T_{FH}) cells.[5-7] T_{FH} cells belong to a subset of CD4$^+$ T cells. After antigen stimulation, CD4$^+$ naïve T cells differentiate into T helper type 1 (T_H1), type 2 (T_H2), interleukin (IL)-17-producing helper T (T_H17) cells, inducible regulatory T (iT_{Reg}) cells, or T_{FH} cells.[8,9] Of these, T_{FH} cells are defined as CD4$^+$ T cells that express chemokine C-X-C motif receptor 5 (CXCR5), inducible T-cell co-stimulator (ICOS), programmed cell death protein 1 (PD1), B-cell lymphoma 6 (BCL-6), and IL-21.[8,9]

In AITL, chemokine CXCL13 is considered to be a tumor marker.[1,10,11] CXCL13, together with its cognate receptor CXCR5 (i.e., the CXL13-CXCR5 axis), plays an essential role in the formation of germinal centers in the secondary lymphoid organs.[12,13] FDCs in such lymphoid organs express CXCL13.[14-17] FDCs form a well-organized meshwork in the light zone of germinal centers and express antigen-antibody complex on their cell surface, thereby mediating the positive selection of B cells with high-affinity antibodies.[16,17] FDCs

Received: May 15, 2015
Revised : June 10, 2015
Accepted: June 30, 2015

[1)]Department of Pathology, Mito Saiseikai General Hospital, Mito, Japan Departments of [2)]Pathology and [3)]Hematology, Mito Medical Center, National Hospital Organization, Ibaraki, Japan
[4)]Department of Medical Hygiene, Kochi Gakuen College, Kochi, Japan
[5)]Division of Tumor Pathology, Department of Pathological Sciences, Faculty of Medical Sciences, University of Fukui, Eiheiji, Japan
[6)]Department of Pathology, Omachi Municipal General Hospital, Omachi, Japan
[7)]Department of Pathology, Tsukuba University Hospital, Tsukuba, Japan
[8)]Department of Pathology, Tokai University School of Medicine, Isehara, Japan
Corresponding author: Dr. Haruo Ohtani, Department of Pathology, Mito Saiseikai General Hospital, 3-3-10 Futabadai, Mito 311-4198, Japan
E-mail: 311 serenity@gmail.com

secrete CXCL13 to chemo-attract $CXCR5^+$ follicular B cells and $CXCR5^+$ T_{FH} cells. Agostinelli *et al*. described that CXCL13 was also expressed in FDCs in AITL.[4] Considering this complicated situation, the present study revisited CXCL13 to perform the exact cell identification of $CXCL13^+$ cells in AITL in comparison to normal germinal centers. We show here that CXCL13 is expressed mainly in FDCs and also in neoplastic T cells, and that the FDC meshwork in AITL harbors neoplastic cells and B cells. CXCR5, counter-receptor of CXCL13, is expressed by neoplastic cells. We discuss the possible significance of these findings in relation to the histogenesis of AITL.

MATERIALS AND METHODS

Formalin-fixed, paraffin-embedded tissue samples were used. In all lymphoma cases, tissue samples were obtained at the initial diagnosis, including 33 cases of AITL (mean age 67.4 ± 13.3 years, males: females (M:F) = 18:15) and six cases of AITL-like PTCL (mean age 69.8 ± 10.1 years, M:F = 3:3).[4] All lymphoma cases were of nodal type. The diagnosis of AITL was performed as described by the WHO classification.[1] All AITL-like PTCLs in the present study showed less developed HEVs than AITL, retaining the FDC meshwork.

For the normal controls, both secondary lymphoid organs and tertiary lymphoid tissues were used. The secondary lymphoid organs (18 cases, mean age 46.8 ± 22.1 years, M:F = 10:8) included seven cases of chronic tonsillitis and 11 cases of reactive hyperplasia of the lymph nodes. Tertiary lymphoid tissues (12 cases, mean age 67.4 ± 11.6 years, M:F = 8:4) included four cases of chronic cholecystitis, two cases of nodular lymphoid hyperplasia of the lung, and one case each of chronic urocystitis, chronic thyroiditis, inflammatory pseudotumor of the lung, chronic sclerosing sialoadenitis, chronic esophagitis, and diverticulitis. These control tissues were obtained by surgical resection.

For conventional immunohistochemistry, the second-step immunoperoxidase method was used as described previously.[18] Antigen retrieval was performed by immersing glass slides in 10 mM Tris buffer/1 mM EDTA at 95℃ for 60 min. For CD21 immunostaining, DAKO S-1700 was used for antigen retrieval. After the blocking of non-specific reactivity by Protein Block (DAKO, Glostrup, Denmark), primary antibodies were applied (Table 1). After quenching the endogenous peroxidase activity with 3% H_2O_2 for 5 min, Envision plus (DAKO) was applied as a secondary antibody. 3,3'-Diaminobenzidine tetrahydrochloride was used as a chromogen.

For immunofluorescent staining, all of the primary antibodies were applied overnight. Alexa Fluor (350, 488, or 555)-labeled anti-mouse, rabbit, or goat immunoglobulin antibodies (raised in donkey) were used as the secondary antibodies (diluted at 1:100, applied for 30 min) (Molecular Probes; Thermo Fisher Scientific Inc., Waltham, MA, USA). For the double- or triple-immunofluorescent method, two or three different kinds of primary or secondary antibodies were applied in a mixture. 4',6-Diamidino-2-phenylindole (Molecular Probes) was used for nuclear counterstaining. All of the fluorescent images were captured using a digital camera (DS-5M; Nikon, Tokyo, Japan) equipped on a fluorescent microscope (E800; Nikon). For double- and triple-immunofluorescent microscopy, each fluorescent image was manually merged on a computer using PaintShop Photo Pro X3 (Corel, Ottawa, Canada). Detailed analyses of the fluorescent images were performed by confocal laser scanning microscopy (Leica SP5; Wetzlar, Germany).

For the negative control, isotype-matched, control mouse monoclonal antibodies (DAKO) or normal rabbit or goat immunoglobulins were used. These control staining samples showed no significant immunoreactivity.

The present study was approved by the ethics committees of the institutes from which the materials were obtained.

Table 1. List of primary antibodies used in the present research*

Antibody	Concentration used	Source
Goat polyclonal anti-human CXCL13	2 μg/mL (2 μg/mL)	R&D
Rabbit polyclonal anti-human CXCR5	4 μg/mL (20 μg/mL)	GeneTex Inc.
Rabbit polyclonal anti-CD3	1:200 (1:40)	DAKO
Mouse monoclonal anti-CD4 (clone 1F6)	1:40 (1:5)	Abcam
Mouse monoclonal anti-CD20 (clone L26)	1:1,000 (1:40)	DAKO
Mouse monoclonal anti-CD21 (clone 1F8)	1:100 (1:50)	DAKO
Mouse monoclonal anti-FDC (clone CAN.42)	1:50 (1:10)	DAKO
Rabbit monoclonal anti-CD19 (clone EPR5906)	(1:20)	Abcam

The text in parentheses indicates the concentration used for immunofluorescent microscopy. Incubation time was 45 min for the single-labeling immunoperoxidase method and overnight for the immunofluorescent method. R&D, Minneapolis, MN, USA; GeneTex Inc., Irvine, CA, USA; DAKO, Glostrup, Denmark; Abcam, Cambridge, MA, USA
*Primary antibodies used only for diagnostic purposes are not included here.

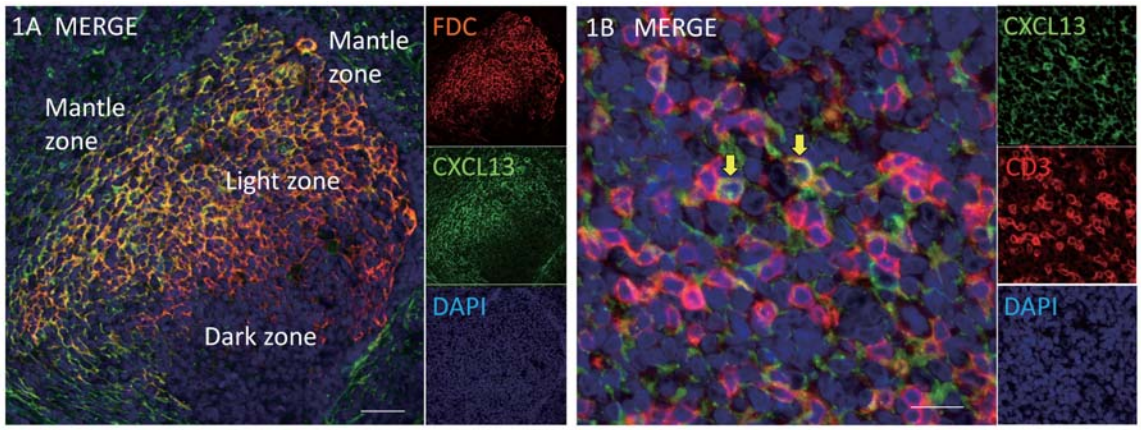


Fig. 1. Double-labeling immunofluorescent microscopy to identify CXCL 13^+ cells in the normal germinal center (tonsil). (***1A***) Most CXCL13 (*green*)-positive cells were follicular dendritic cells (anti-FDC, *red*). Double-positive cells are expressed in *yellow*. (***1B***) T cells (anti-CD3, *red*) are infrequently positive for CXCL13 (*green*) (*arrows*). Scale bars: (*1A*) 50 μm and (*1B*) 20 μm.

RESULTS

Identification of CXCL13$^+$ cells as mainly FDCs and partly T_{FH} cells in the normal lymphoid tissues

Previous reports described that CXCL13 was localized in either T_{FH} cells[10,11] or FDCs[14-17] in the normal lymphoid tissues. Single-labeling immunohistochemistry showed that CXCL13 was expressed as a characteristic meshwork pattern in the light zone of germinal centers in all cases except one (*vide infra* for details). Double-immunofluorescent microscopy clearly showed the co-localization of CXCL13 and FDC markers (Fig. 1A), indicating that CXCL13 was mainly expressed in FDCs. The co-expression of CXCL13 and CD3 (or CD4) was observed infrequently in the germinal centers (Fig. 1B). Since T_{FH} cells are a major component of follicular T-cells,[19] this indicated the infrequent occurrence of CXCL13$^+$ T_{FH} cells. The exception was one case of tertiary lymphoid tissues (chronic thyroiditis), in which CXCL13 was expressed mainly in lymphocytes. CD83$^+$ dendritic cells (DCs) (conventional or classical DCs) in the paracortex also expressed CXCL13 (Supplementary data).

CXCL13 is expressed mainly in FDCs and to various degrees in neoplastic cells in AITL

Of 33 cases of AITL, 32 were of the "classical type" (pattern 2 or 3), and one belonged to pattern 1.[1] Single-labeling immunohistochemistry revealed that CXCL13 was expressed in either a meshwork (Fig. 2A) or a perinuclear dot pattern (Fig. 2B). In 23 of 33 AITL cases, double-immunofluorescent microscopy was performed, which revealed the following findings. In areas with a meshwork pattern, co-localization of CXCL13 and FDC markers was observed (Fig. 2C), confirming that FDCs expressed CXCL13. This also showed a similarity between the FDC meshwork in AITL (Fig. 2C) and the normal germinal center (Fig. 1A). In areas with a perinuclear dot pattern, co-localization of CXCL13 and CD3 (or CD4) was demonstrated (Fig. 2D), indicating that CXCL13 is expressed in neoplastic cells. Combining the results of single- and double-labeling data, we judged that CXCL13$^+$ cells were mainly FDCs associated with various degrees of CXCL13$^+$ neoplastic cells in 28 of 33 AITL cases (all classical type [pattern 2 or 3]). In the other five cases (including one case with pattern 1), CXCL13$^+$ cells were mainly neoplastic cells without remarkable reactivity in FDCs (note that the FDC meshwork was observed in all cases examined). The FDC meshwork in AITL was usually irregular in shape and larger than the normal germinal centers.[20] The cytoplasmic processes of FDCs may be less intensely labeled with CXCL13, occasionally making them difficult to identify as FDCs. Therefore, care should be taken for the presence of cytoplasmic processes to identify FDCs.

Among six AITL-like PTCL cases, CXCL13 was localized in both FDCs and neoplastic cells in three cases, or mainly in neoplastic cells without significant expression in FDCs in the other three cases (data not shown).

CXCL13$^+$ cells are concentrated in FDC meshwork irrespective of the cell source

We next focused our attention on five cases of AITL and three cases of AITL-like PTCL in which mainly neoplastic cells expressed CXCL13. Double-labeling immunofluorescent microscopy revealed that CXCL13$^+$ neoplastic cells were abundantly distributed in and around the FDC meshwork in

six of eight cases (Fig. 2E). Therefore, it was clear that $CXCL13^+$ cells were densely concentrated in the FDC meshwork irrespective of the $CXCL13^+$ cell types in AITL and AITL-like PTCL cases.

FDC meshwork in AITL harbors neoplastic cells and B cells

To explore how neoplastic T cells or B cells were distributed among the FDC meshwork in AITL, we conducted triple-labeling immunofluorescent microscopy for CD3, CXCL13, and CD20. In the normal lymphoid tissues (one case of lymph node and four tonsillitis cases), this triple-color method revealed that both follicular B cells and T cells (mainly T_{FH} cells[19]) were enmeshed among FDCs in the light zone of the germinal centers (Fig. 3A). We next analyzed 10 representative AITL cases, in which CXCL13 was expressed mainly in FDCs. Although the structure of the FDC meshwork in AITL differed from that of the normal germinal centers, the same three components (T cells, B cells, and FDCs) were identified in the FDC meshwork in AITL with T-cell dominance (Fig. 3B).

We next analyzed whether or not neoplastic T cells were included in the FDC meshwork in AITL. Single-labeling immunohistochemistry revealed that the FDC meshwork in AITL harbored neoplastic cells with large, atypical nucleus (Fig. 4A). The normal germinal centers lacked such atypical cells (Fig. 4B). Close observation by the triple-color method revealed that both $CD3^+$ neoplastic cells and $CD20^+$ B cells were closely juxtaposed to $CXCL13^+$ FDCs with neoplastic cells as a major component (Fig. 4C). High-resolution images were obtained by confocal laser scanning microscopy, which showed that neoplastic $CD3^+$ cells with atypical nucleus (shown in *green*) were in close contact with $CXCL13^+$ FDCs (shown in *red*) (Fig. 4D). The data so far indicated that the FDC meshwork in AITL harbored significant numbers of neoplastic T cells as well as B cells. This indicates that the FDC meshwork in AITL retains a similarity to normal germinal centers.

This close contact between neoplastic cells and FDCs in AITL (Fig. 4C & 4D) was unexpected. Therefore, normal germinal centers were analyzed by the same method, where T cells (mainly T_{FH} cells) were again in close contact with FDCs (Fig. 4E). Our observations suggest certain interactions between FDCs and T cells (for details, see Discussion).

Expression of CXCR5 in AITL

We next analyzed the expression of CXCR5, the cognate receptor of CXCL13. In the normal tissue, CXCR5 is reported to be expressed in follicular B cells and T_{FH} cells.[17] Double-immunofluorescent microscopy in the normal lymphoid follicles showed, therefore, that $CXCR5^+$ cells were specifically distributed in the germinal centers closely associated with $CXCL13^+$ FDCs (Fig. 5A). This is in accordance with the ligand-receptor relationship. In AITL (20 cases), single-labeling immunohistochemistry revealed that $CXCR5^+$ cells were distributed diffusely in neoplastic tissues (Fig. 5B & 5C). Double-immunofluorescent microscopy was performed in five AITL cases and one AITL-like PTCL case, in which CXCL13 was mainly expressed in FDCs. This revealed that $CXCR5^+$ cells were distributed in and around the $CXCL13^+$ FDC meshwork (Fig. 5D). The data here (Fig. 5A vs. Fig. 5D) again indicated a similarity between the FDC meshwork and the normal germinal centers.

DISCUSSION

The present study analyzed AITL from the viewpoint of CXCL13 expression and formation of the FDC meshwork. Since the cell identification in AITL is complicated, multi-color immunofluorescent microscopy was adopted, to reveal the following findings: 1) concentrated expression of CXCL13 in FDC meshwork areas in AITL, 2) similarities between the FDC meshwork in AITL and normal germinal centers, and 3) a close relationship between CXCL13 and its cognate receptor, CXCR5, in both AITL and normal germinal centers. Normal T_{FH} cells play important roles in the formation and maintenance of germinal centers and B-cell differentiation.[9] Therefore, our results based on the analyses of immuno-architecture are consistent with a view that neoplastic cells in AITL retain a certain level of T_{FH}-cell functions.

We first need to discuss the much wider expression of CXCL13 in FDCs than is currently considered and the accumulation of $CXCL13^+$ cells in and around FDC meshwork areas, irrespective of the cell types that express CXCL13. In this sense, some differences are noted between our data and previous observations.[10,11] As stated in the Results, it is likely that not all FDCs express CXCL13 in the whole cytoplasm, making cytoplasmic processes occasionally less visible. This may cause FDCs to be recognized as tumor cells. This is why we adopted double-immunofluorescent microscopy for the identification of $CXCL13^+$ cells.

We next confirmed the presence of a similarity between the FDC meshwork in AITL and the normal germinal centers. In both, FDCs frequently express CXCL13, and both harbored B cells and T cells. The distribution of CXCR5, the cognate receptor of CXCL13, further supports this similarity. This similarity also suggests that the CXCL13-CXCR5 axis may be involved in the formation of the FDC meshwork in AITL, in a similar way as in normal germinal centers.[12,13] Differences were also noted; for example, in AITL, the FDC meshwork was disorganized structurally, and it harbored abundant neoplastic T cells, contrasted by B-cell dominance in the normal germinal centers. The ubiquitous distribution

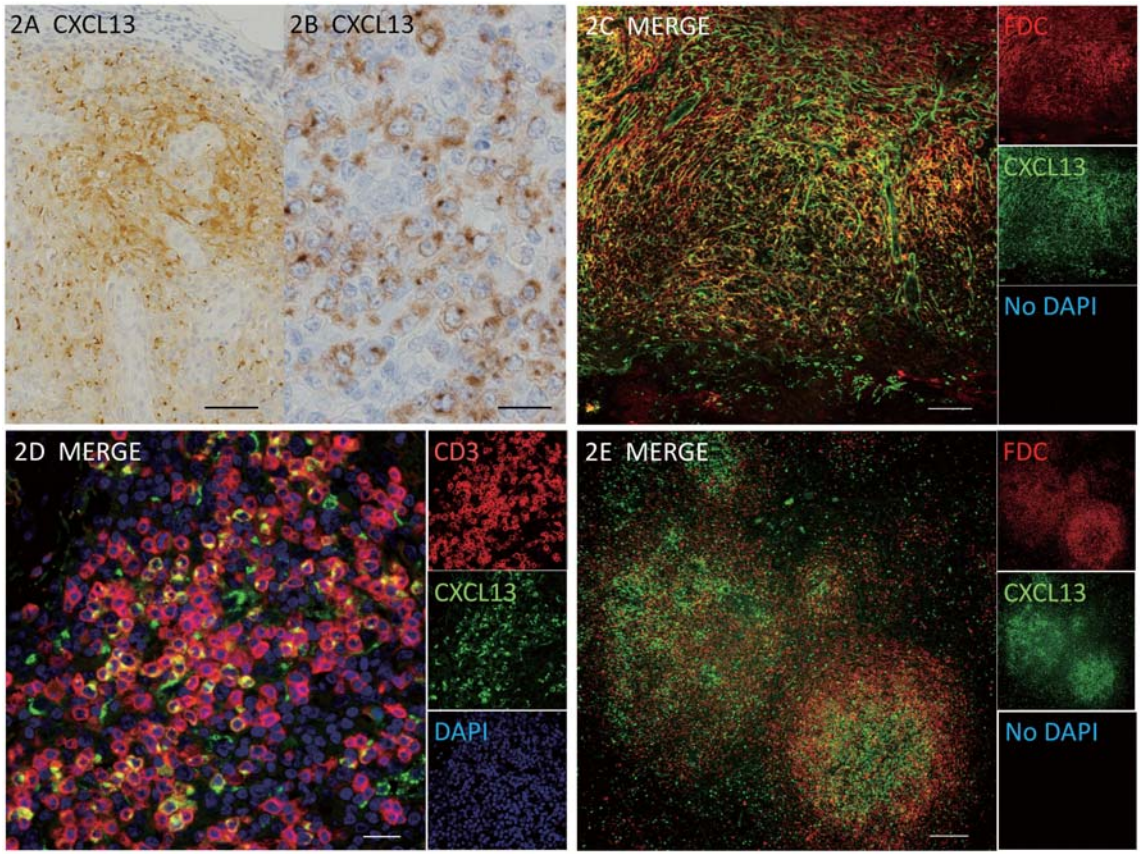


Fig. 2. CXCL13 in angioimmunoblastic T-cell lymphoma. (***2A*** & ***2B***) Single-labeling immunohistochemistry for CXCL13 reveals a meshwork pattern (*2A*) and perinuclear dot pattern (*2B*). (***2C*** & ***2D***) Double-labeling immunofluorescent microscopy revealed that CXCL13 (*green*)-positive cells were mainly follicular dendritic cells (FDCs) (*red*) in areas of meshwork pattern (*2C*) and mainly neoplastic cells (anti-CD3, *red*) in areas of perinuclear dot pattern (*2D*). (***2E***) $CXCL13^{+}$ neoplastic cells (*green*) were densely distributed in and around the FDC meshwork (anti-FDC, *red*) in a case where mainly neoplastic cells expressed CXCL13. Scale bars: (*2A*) 40 μm, (*2B*) 20 μm, (*2C*) 100 μm, (*2D*) 20 μm, and (*2E*) 200 μm.

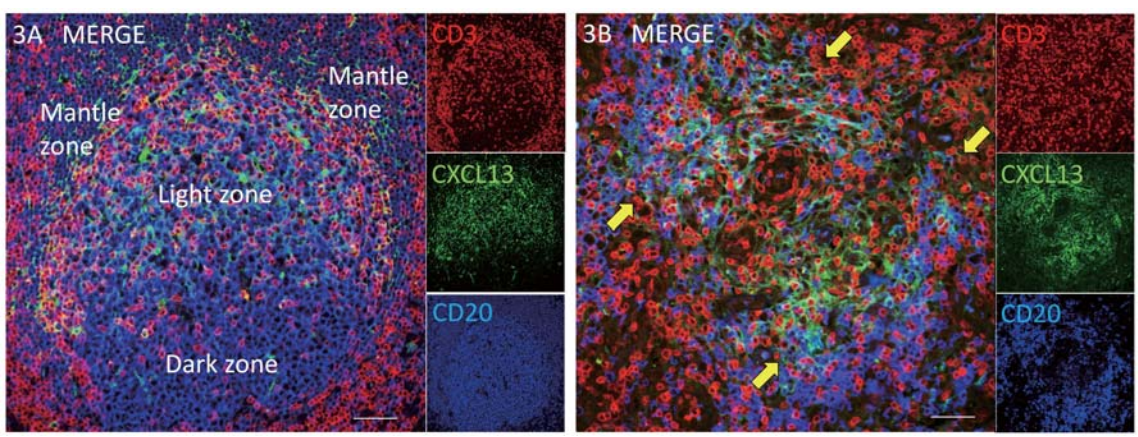


Fig. 3. Triple-labeling immunofluorescent microscopy for CD3 (*red*), CXCL13 (*green*), and CD20 (*blue*). Note a well-organized structure of the normal germinal center (*3A*) and a disorganized follicular dendritic cell meshwork in angioimmunoblastic T-cell lymphoma (*3B*, indicated by *yellow arrows*). The basic cell components are similar. Scale bars: (*3A* & *3B*) 50 μm.

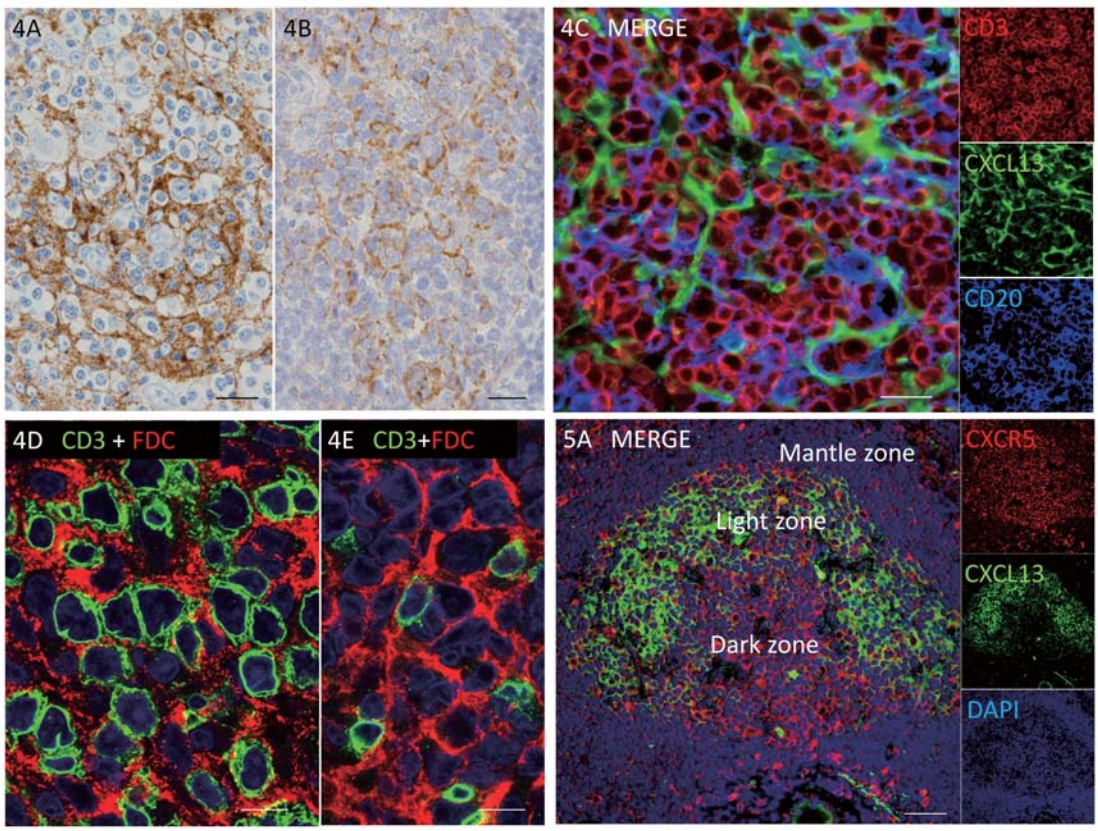


Fig. 4. Relationship between follicular dendritic cells (FDCs) and neoplastic cells in angioimmunoblastic T-cell lymphoma (AITL). (***4A*** & ***4B***) Single labeling for FDCs shows that large neoplastic cells are enmeshed in the FDC meshwork in AITL (*4A*, anti-FDC), which is cytologically contrasted by the normal germinal center (*4B*, anti-CD21, tonsillitis). (***4C***) Triple-labeling method confirmed that $CD3^+$ T cells (*red*) and $CD20^+$ B cells (*blue*) are in close contact with $CXCL13^+$ FDCs (*green*) in AITL. (***4D*** & ***4E***) Confocal laser scanning microscopy demonstrates that the FDC meshwork (*red*) harbors atypical neoplastic T cells (*green*) in AITL (*4D*) and T cells (*green*) in normal germinal centers (*4E*, tonsillitis) (anti-FDC, *red*). Scale bars: (*4A* & *4B*) 25 μm, (*4C*) 20 μm, and (*4D* & *4E*) 10 μm.

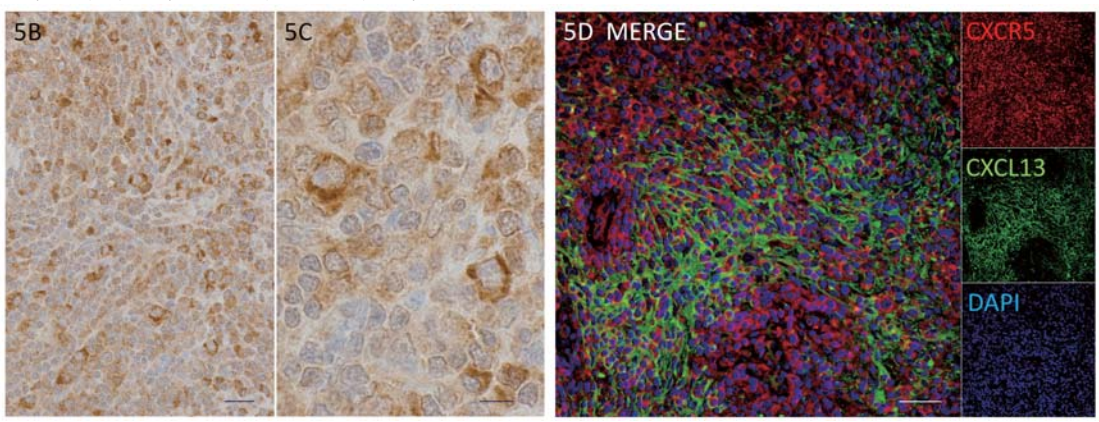


Fig. 5. Close spatial relationship between CXCL13 and its cognate receptor CXCR5. (***5A***) In normal lymphoid tissues, $CXCR5^+$ cells (*red*) are localized in the whole germinal center together with the $CXCL13^+$ follicular dendritic cell (FDC) meshwork (green). (***5B*** & ***5C***) In angioimmunoblastic T-cell lymphoma (AITL), CXCR5 (*brown*) is diffusely expressed in neoplastic cells (*5B*), with larger cells more strongly positive than smaller ones (*5C*). (***5D***) Double-labeling method confirmed that $CXCR5^+$ cells (*red*) are located in and around the $CXCL13^+$ FDC meshwork (*green*) in AITL. Scale bars: (*5A*, *5B* & *5D*) 50 μm and (*5C*) 10 μm.

of $CXCR5^+$ cells in AITL was also different from the concentration of $CXCR5^+$ cells in the normal germinal centers. The similarities between the FDC meshwork in AITL and normal germinal centers described above support the current histogenesis theory, which considers that AITL originates from T_{FH} cells because neoplastic cells may contribute to the formation of the FDC meshwork so as to accumulate B cells in those areas as the normal T_{FH} cells do in the germinal centers.[8,9] This could explain the proliferation of B cells and plasma cells, as well as polyclonal gammopathy, in AITL. Recent gene expression analyses showed that the participation of B cells in AITL is a favorable prognostic factor.[7,21] Taking these findings together, it is possible that neoplastic cells in AITL retain a certain level of T_{FH}-cell function. In other words, we may be able to consider that AITL is "a functioning tumor" of T_{FH} cells so as to form the FDC meshwork in tumor tissue.

As expected from its localization in the normal germinal centers, CXCR5 was reported to be expressed in follicular lymphoma,[22] and the host genetic variation in CXCR5 has a role in lymphomagenesis, particularly for follicular lymphoma.[23] A recent study also suggested the possibility of targeting CXCR5 in B-cell non-Hodgkin lymphoma.[24]

The close contact between neoplastic T cells (or normal T_{FH} cells) and FDCs suggests a functional relationship between these two cells. It was suggested that T_{FH} cells may require ongoing signals from B cells and FDCs to maintain the T_{FH} program,[9] which may be relevant to the present observations.

Recent studies revealed that hyperplastic follicles in pattern 1 AITL are different from reactive follicles.[25,26] The relationship between FDCs in these hyperplastic follicles and the FDC meshwork in pattern 2 or 3 will be an important theme of future studies, since detailed analyses were not performed in the present study due to the limited number of cases with pattern 1.

To conclude, the present study clarified the structure of the FDC meshwork in AITL to reveal similarities to normal germinal centers, based on analyses of the immunoarchitecture. The results suggest that AITL could be regarded as "a functioning tumor" of T_{FH} cells. Further analyses will be required.

ACKNOWLEDGMENTS

The present study was supported by a National Hospital Organization Collaborative Clinical Research Grant (Mito Medical Center), Japan, and 'Nanotechnology Network Japan' from the Ministry of Education, Culture, Sports, Science and Technology, Japan (National Institute for Materials and Science, Tsukuba). We also wish to acknowledge technical assistance from Dr. Xianglan Li and Ms. Shoko Kajiwara (National Institute for Materials and Science [NIMS]-Leica Bioimaging Laboratory) for the confocal laser scanning microscopy.

DISCLOSURE STATEMENT

The authors declare that there are no conflicts of interest regarding the present paper.

REFERENCES

1 Dogan A, Gaulard P, Jaffe ES, Ralfkiaer E, Müller-Hermelink HK: Angioimmunoblastic T-cell lymphoma. In: Swerdlow SH, Campo E, Harris NL, Jaffe ES, Pileri SA, *et al*. (eds): World Health Organization Classification of Tumours, WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues. 4th ed, Lyon, International Agency for Research on Cancer (IARC), pp.309-311, 2008

2 Piccaluga PP, Agostinelli C, Tripodo C, Gazzola A, Bacci F, *et al*.: Peripheral T-cell lymphoma classification: the matter of cellular derivation. Expert Rev Hematol 4:415-425, 2011

3 Jaffe ES, Nicolae A, Pittaluga S: Peripheral T-cell and NK-cell lymphomas in the WHO classification: pearls and pitfalls. Mod Pathol 26:S71-S87, 2013

4 Agostinelli C, Hartmann S, Klapper W, Korkolopoulou P, Righi S, *et al*.: Peripheral T cell lymphomas with follicular T helper phenotype: a new basket or a distinct entity ? Revising Karl Lennert's personal archive. Histopathology 59:679-691, 2011

5 Rodríguez-Pinilla SM, Atienza L, Murillo C, Pérez-Rodríguez A, Montes-Moreno S, *et al*.: Peripheral T-cell lymphoma with follicular T-cell markers. Am J Surg Pathol 32:1787-1799, 2008

6 de Leval L, Rickman DS, Thielen C, Reynies AD, Huang YL, *et al*.: The gene expression profile of nodal peripheral T-cell lymphoma demonstrates a molecular link between angioimmunoblastic T-cell lymphoma (AITL) and follicular helper T (T_{FH}) cells. Blood 109:4952-4963, 2007

7 Iqbal J, Weisenburger DD, Greiner TC, Vose JM, McKeithan T, *et al*.: Molecular signatures to improve diagnosis in peripheral T-cell lymphoma and prognostication in angioimmunoblastic T-cell lymphoma. Blood 115:1026-1036, 2010

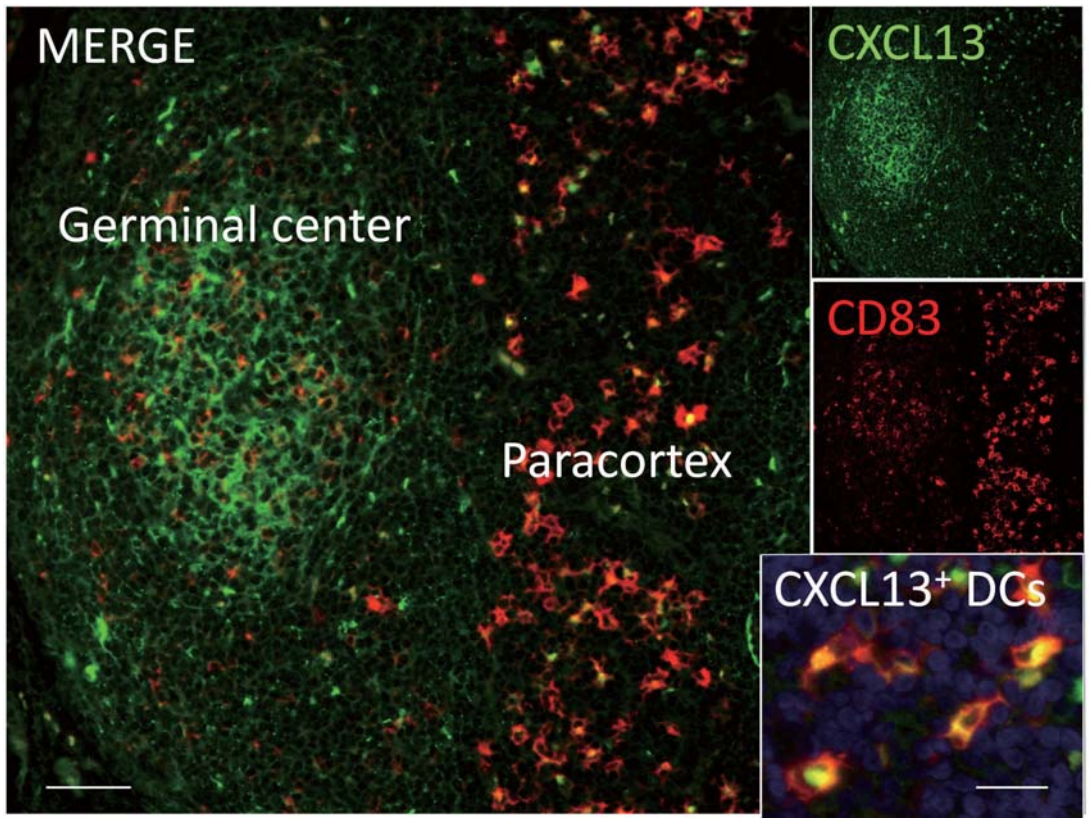
8 Crotty S: T follicular helper cell differentiation, function, and roles in disease. Immunity 41:529-542, 2014

9 Ueno H, Banchereau J, Vinuesa CG: Pathophysiology of T follicular helper cells in humans and mice. Nat Immunol 16:142-152, 2015

10 Dupuis J, Boye K, Martin N, Copie-Bergman C, Plonquet A, *et al*.: Expression of CXCL13 by neoplastic cells in angioimmunoblastic T-cell lymphoma (AITL): a new diagnostic marker providing evidence that AITL derives from follicular helper T cells. Am J Surg Pathol 30:490-494, 2006

11 Grogg KL, Attygalle AD, Macon WR, Remstein ED, Kurtin PJ, *et al*.: Expression of CXCL13, a chemokine highly upregulated in germinal center T-helper cells, distinguishes angioimmunoblastic T-cell lymphoma from peripheral T-cell lymphoma, unspecified.

Mod Pathol 19:1101-1107, 2006
12 Legler DF, Loetscher M, Roos RS, Clark-Lewis I, Baggiolini M, *et al*.: B cell-attracting chemokine 1, a human CXC chemokine expressed in lymphoid tissues, selectively attracts B lymphocytes via BLR1/ CXCR5. J Exp Med 187:655-660, 1998
13 Ansel KM, Ngo VN, Hyman PL, Luther SA, Förster R, *et al*.: A chemokine-driven positive feedback loop organizes lymphoid follicles. Nature 406:309-314, 2000
14 Vissers JL, Hartgers FC, Lindhout E, Figdor CG, Adema GJ: BLC (CXCL13) is expressed by different dendritic cell subsets *in vitro* and *in vivo*. Eur J Immunol 31:1544-1549, 2001
15 Vermi W, Lonardi S, Bosisio D, Uguccioni M, Danelon G, *et al*.: Identification of CXCL13 as a new marker for follicular dendritic cell sarcoma. J Pathol 216:356-364, 2008
16 El Shikh ME, Pitzalis C: Follicular dendritic cells in health and disease. Front Immunol 3:1-18, 2012
17 Aguzzi A, Kranich J, Krautler NJ: Follicular dendritic cells: origin, phenotype, and function in health and disease. Trends Immunol 35:105-113, 2014
18 Ohtani H, Jin Z, Takegawa S, Nakayama T, Yoshie O: Abundant expression of CXCL9 (MIG) by stromal cells that include dendritic cells and accumulation of $CXCR3^+$ T cells in lymphocyte-rich gastric carcinoma. J Pathol 217:21-31, 2009
19 Ramiscal RR, Vinuesa CG: T-cell subsets in the germinal center. Immunol Rev 252:146-155, 2013
20 Carbone A, Gloghini A: Follicular dendritic cell pattern in early lymphomas involving follicles. Adv Anat Pathol 21:260-269, 2014
21 Iqbal J, Wright G, Wang C, Rosenwald A, Gascoyne RD, *et al*.: Gene expression signatures delineate biological and prognostic subgroups in peripheral T-cell lymphoma. Blood 123:2915-2923, 2014
22 Husson H, Freedman AS, Cardoso AA, Schultze J, Munoz O, *et al*.: CXCL13 (BCA-1) is produced by follicular lymphoma cells: role in the accumulation of malignant B cells. Br J Haematol 119: 492-495, 2002
23 Charbonneau B, Wang AH, Maurer MJ, Asmann YW, Zent CS, *et al*.: CXCR5 polymorphisms in non-Hodgkin lymphoma risk and prognosis. Cancer Immunol Immunother 62:1475-1484, 2013
24 Panjideh H, Müller G, Koch M, Wilde F, Scheu S, *et al*.: Immunotherapy of B-cell non-Hodgkin lymphoma by targeting the chemokine receptor CXCR5 in a preclinical mouse model. Int J Cancer 135:2623-2632, 2014
25 Rodriguez-Justo M, Attygalle AD, Munson P, Roncador G, Marafioti T, *et al*.: Angioimmunoblastic T-cell lymphoma with hyperplastic germinal centers: a neoplasia with origin in the outer zone of the germinal center ? Clinicopathological and immunohistochemical study of 10 cases with follicular T-cell markers. Mod Pathol 22:753-761, 2009
26 Tan LH, Tan SY: Aberrant immunoarchitecture distinguishes hyperplastic germinal centers in pattern 1 angioimmunoblastic T-cell lymphoma from reactive follicles. Hematol Oncol 32: 145-154, 2014



Supplementary data. In the normal lymphoid tissue (lymph node), double-labeling immunofluorescent microscopy confirms that significant numbers of $CD83^+$ (*red*) mature, conventional dendritic cells in the paracortex co-express CXCL13 (*green*), which is clearly demonstrated at higher magnification (double positivity in *yellow* in the lower right inset). Scale bars: 50 µm (main, merged figure) and 20 µm (lower right inset).