T-cell/histiocyte-rich large B-cell lymphoma

Stefania Pittaluga and Elaine S. Jaffe

Hematopathology Section, Laboratory of Pathology, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, MD, USA. E-mail: elainejaffe@nih.gov. *doi:10.3324/haematol.2009.016931*

(Related Original Article on pag. 440)

T-cell/histiocyte-rich large B-cell lymphoma (THRLBCL) can be used as a paradigm to highlight the difficulties in defining specific subtypes of diffuse large B-cell lymphoma. Since the first recognition of certain large B-cell lymphomas in which the tumor cell content was dramatically outnumbered by reactive T cells or less often histiocytes, this descriptive term and related terms have been used to characterize a variety of lymphoma subtypes, and different studies have varied in the criteria used for this designation. To further complicate the issue, THRLBCL shares several morphological and immunophenotypic similarities with nodular lymphocyte-predominant Hodgkin's lymphoma (NLPHL). Although the clinical presentation and behavior of these two diseases are generally quite distinct, some cases show overlapping features, suggesting a close biological relationship. The finding that both lymphomas may occur concurrently or subsequently in the same patient and/or families further supports the hypothesis that at least a subset is closely related. Finally, both NLPHL and THRLBCL may progress to more typical diffuse large B-cell lymphoma, once the neoplastic cells sheet out and the stroma is no longer the predominant component.

Historical background

The original term of "T-cell-rich B-cell lymphoma" was introduced to describe a variety of B-cell lymphomas with a prominent T-cell reaction mimicking T-cell lymphoma.[1] The presence of less than 10% (sometime as low as 1%) of usually large, atypical B cells in a background rich in T cells was the unifying feature of the diagnosis. B-cell lymphomas with a prominent T-cell reaction ranged from low-grade lymphomas such as chronic lymphocytic leukemia[1] and follicular lymphoma to diffuse large B-cell lymphomas.[1-3] These were important diagnostic contributions that emphasized the utility of immunohistochemistry, and in some cases molecular studies,[4] for the correct identification of the neoplastic population in an unusually rich reactive milieu. However, at this stage the emphasis was on the distinction from peripheral T-cell lymphoma, and not on the delineation of a new disease entity. Indeed, it appeared that this host reaction could be associated with a variety of B-cell neoplasms.

Based on early immunohistochemical studies of the microenvironment, Macon *et al.* suggested that cytokines such as interleukin-4 could play a role in the histogenesis of this lymphoma.[5] The same group later characterized the T-cell infiltrate as being predominantly composed of non-activated $CD8^+$/TIA-1^+/granzyme B^- T cells, and speculated that it might have been less effective in mediating a host anti-tumor response.[6] Delabie *et al.* identified a distinct subgroup predominantly rich in non-epithelioid histiocytes rather than T cells with distinctive clinicopathological features (the so-called "histiocyte-rich B-cell lymphoma").[7] Their study also stressed the similarities and possible relatedness with NLPHL, which is likewise characterized by relatively rare neoplastic cells in an abundant non-neoplastic cellular environment. They described distinctive clinical features including a high risk of bone marrow involvement, hepatosplenomegaly and an aggressive clinical course, which was observed by others,[8,9] and confirmed in a subsequent larger study.[10] However, these observations were not verified in other studies, probably reflecting either the biological heterogeneity still present within this group of lymphomas or variability in diagnostic criteria.[11,12] Based on these uncertainties, THRLBCL was included only as a histological variant of diffuse large B-cell lymphoma in the World Health Organization (WHO) classification of 2001.[13]

In an effort to further understand the biology and underlying molecular events, and to define the relationship between THRLBCL and NLPHL, including the diagnostic criteria and therapeutic implications, an international workshop was held on "grey zone lymphoma" during the Fifth International Congress on Hodgkin's Lymphoma.[14] It was recognized that precise criteria for the diagnosis of THRLBCL were not available. Moreover, it was appreciated that NLPHL could show progression to a process closely mimicking THRLBCL, but that the relationship of *de novo* THRLBCL to secondary THRLBCL was still unknown.

In the subsequent years, several studies focused on the relationship between NLPHL and THRLBCL, the phenotype and genetic characterization of the neoplastic B cells in both entities, as well as the composition of the non-neoplastic background cells.[6,15,16] The morphology, phenotype and genetics of the neoplastic B cells supported a germinal center B-cell derivation with expression of BCL-6, but there was relatively infrequent expression of CD10 and, in most cases, absence of both BCL-2 protein and the t(14;18).[15-17] Epithelial membrane antigen, a marker associated with lymphocyte-predominant cells, (formerly known as L&H cells or popcorn cells) was seen in a subset of cases.[16] Variations in immunophenotype correlated with variations in morphology, and suggested that THRLBCL as currently defined is not a homogeneous disease entity.[16] Nevertheless, none of these markers helped in the differential diagnosis between THRLBCL and NLPHL, and the interest shifted towards the analysis of transcription factors involved in B-cell, T-cell and macrophage differentiation.

An early study focused on the expression of transcription factors in Hodgkin's lymphoma. It was noted that PU.1 was expressed in NLPHL, while it was lacking in THRLBCL, suggesting that it could be a useful diagnostic marker.[18,19] However, other studies did not confirm this initial observation.[20] Additional key factors in this differential diagnosis are based on the cellular composition and char-

acter of the stromal reaction, rather than the neoplastic cells themselves.[9] Typically lymphocyte-predominant cells maintain a stronger association with follicular structures, as represented by small B cells that are predominantly IgD positive, as well as follicular dendritic cells. The follicular T helper cells, with their distinctive functional characteristics and phenotype ($CD4^+$, $CD57^+$, $PD\text{-}1^+$), often rosette the lymphocyte-predominant cells.[21-23]

In contrast, in THRLBCL the infrequent large B cells are seen on a background rich in reactive histiocytes and T cells with CD8 predominating often TIA^+, granzyme B^-,[6] and lacking follicular dendritic meshworks. Nevertheless, cases with overlapping histological and phenotypic features have been described and remain a diagnostic and therapeutic challenge.[24] Additionally, NLPHL, especially in recurrences, can show a loss of follicular B-cell elements and an influx of T cells, with the ultimate picture being histologically indistinguishable from THRLBCL.[25]

In the 2008 WHO classification, THRLBCL is included as a specific subtype of diffuse large B-cell lymphoma.[26] The definition emphasizes a stromal component containing numerous T cells and non-epithelioid histiocytes (Figure 1). However, heterogeneity in the neoplastic large B-cell component is acknowledged; these cells may resemble lymphocyte-predominant cells, centroblasts, or more pleomorphic cells similar to Hodgkin's cells. Whether THRLBCL represents more than one disease entity is still not fully resolved, and is still the subject of ongoing debate and study.

Genetics and gene expression profiling

One of the main challenges in performing gene expression profiling or any genetic studies in cases of NLPHL and THRLBCL comes from the intrinsic difficulty of having so few neoplastic cells (usually < 5%) in a rich reactive background, a fact which requires single cell isolation if one wants to investigate the neoplastic component.

Early genetic studies on isolated lymphocyte-predominant cells established their B-cell identity by identifying clonally rearranged immunoglobulin heavy chain genes with the presence of somatic mutations.[27-29] In addition rearrangements of *BCL6* were detected by fluorescence *in situ* hybridization in about 48% of cases, further supporting a relationship with germinal center B cells.[30] By combining comparative genomic hybridization and microdissection of single neoplastic cells with subsequent DNA amplification by degenerate oligonucleotide-primed polymerase chain reaction (DOP-PCR), Franke *et al.* demonstrated the presence of frequent genomic imbalances in lymphocyte-predominant cells.[31] In an attempt to resolve at a genetic level the relationship between lymphocyte-predominant cells and the neoplastic B cells of previously reported and well characterized THRLBCL, Franke *et al.* used the same technique to compare the overall genetic profile of purified cells from both entities.[32] This study revealed that they shared some distinctive genomic changes. Some of the shared changes were also observed in other B-cell lymphomas (e.g., over-representation of the X chromosome and loss of the short arm of chromosome

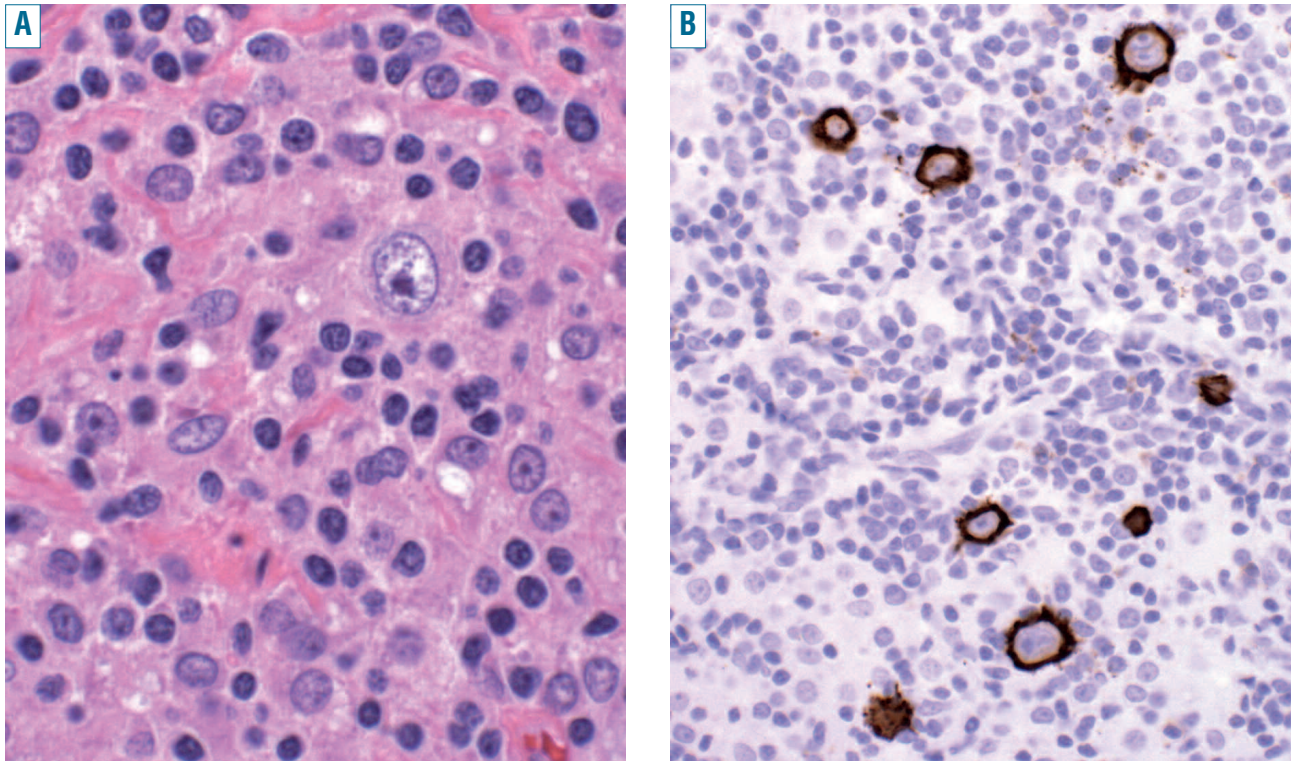

Figure 1. T-cell/histiocyte-rich large B-cell lymphoma. (A) Scattered large neoplastic B-cells are present on a background rich in histiocytes and small T lymphocytes (hematoxylin and eosin). (B) A CD20 stain identifies neoplastic cells, but there are very few small, reactive B cells (CD20 immunohistochemistry).

17). However, the most striking finding was the greater number of genomic alterations and their complexity in NLPHL. These differences were not only related to the overall number of imbalances (gains and losses), but also to their distribution per tumor. The authors hypothesized that these two entities may share a common initial transforming event that occurs in a common shared cell of origin, such as a germinal center B cell. However, their findings suggested early divergence in the evolution of the neoplastic process, and did not support a closer relationship between the two entities. Of interest, the complexity of genomic changes did not correlate with the clinical course in either of these two entities.

More recently gene expression profiling on isolated lymphocyte-predominant cells revealed that these cells are more closely related to Hodgkin-Reed Sternberg (HRS) cells than to germinal center cells and that their expression profile places them at the transition stage between germinal center and memory B cells.[33] When the expression pattern was compared with those of other B-cell lymphomas, NLPHL clustered with classical Hodgkin's lymphoma and THRLBCL, but showed distinguishing features from germinal center reactive cells and all other common types of B-cell lymphomas (follicular lymphoma, Burkitt's lymphoma, and diffuse large B-cell lymphoma, not otherwise specified).[33] Moreover, the gene expression profiling data supported a certain degree of relatedness between NLPHL, THRLBCL, and classical Hodgkin's lymphoma, as all subtypes showed some loss or down-regulation of the B-cell program.

Lymphocyte-predominant cells share significant similarities with the Hodgkin's cells of classical Hodgkin's lymphoma, such as reduced expression of B-cell transcription factors and activation of the nuclear factor-κB and Jak/STAT pathways. However, there are notable differences, such as lack of mutations in the nuclear factor-κB regulating factors IκBα and A20, suggesting that other mechanisms of activation and transformation are probably involved in lymphocyte-predominant cells.[34] Additionally, lymphocyte-predominant cells may escape immune surveillance based on the over-expression of anti-apoptotic genes and down-regulation of pro-apoptotic genes.[33]

Gene expression profiling of THRLBCL has also been investigated when cases were included as part of the histological spectrum of diffuse large B-cell lymphomas. In a study by Monti *et al.* these few cases (8/10) not unexpectedly fell within the "host response" cluster; few known genetic changes were present in this group and a prominent host immune response was detected.[35] However, there was not a complete overlap between histology and the expression pattern, since other diffuse large B-cell lymphomas were also included in the "host response" cluster as well. THRLBCL were not identified nor discussed as a specific subgroup in other gene expression profiling studies, even when the prognostic relevance of "stromal signature" was defined.[36]

A distinct gene expression profile was recently described for THRLBCL when these cases were compared to classical Hodgkin's lymphoma and reactive lymphadenitis; the signature was distinct and revealed overexpression of "interferon pathway and antiviral response" sets of genes (236 probe sets) as well as of genes related to histiocytes/T cells/innate immune response (222 probe sets).[37] In that analysis, although there was a strong influence of the PD-1-dependent pathway, it was not associated with a T helper follicular cell phenotype as shown in other lymphomas and the authors speculated that elevated PD-1 expression in THRLBCL could be induced by other factors such as interferon.

In a study published in this issue of the journal, Van Loo *et al.*[38] exploited the well-known and distinctive characteristics of the microenvironment that surrounds lymphocyte-predominant cells and the neoplastic cells of THRLBCL to investigate whether stromal interactions could play an important role in determining the clinical outcome in the condition, similar to what has been shown in other B-cell lymphomas.[36,39] Their assumption was that the paucity of the neoplastic cells in both lymphomas will not interfere with the analysis of the microenvironment, but that the neoplastic cells may determine how the microenvironment will respond. Thus, they compared the gene expression profile of THRLBCL with that of NLPHL. They focused on THRLBCL cases with clear-cut histology and a prominent histiocytic component, as previously described by this group. They excluded cases with ambiguous overlapping features between these two entities and also excluded cases of lymphocyte-rich classical Hodgkin's lymphoma. As a control, they appropriately chose reactive lymph nodes, which would provide a molecular signature consistent with reactive hyperplasia. The molecular signature that they identified in the NLPHL had a gene expression profile characteristic of B cells, implying that the follicular component seen in typical NLPHL is the predominant feature. Since there was minimal overlap with the signature from the purified lymphocyte-predominant cells, as reported by Brune *et al.*,[33] they concluded that the profile was determined by the background B cells, and not the neoplastic cells. In contrast, the molecular signature of the THRLBCL was dominated by an interferon-dependent pathway, similarly to the data from Chetaille *et al.* (e.g. STAT-1, ICAM-1, CD64, and CXCL10),[37] and in particular included genes associated with macrophages and dendritic cells such as those for CCL8 and indoleleamine 2,3-dioxygenase (IDO), both known to be induced by interferon. IDO is a tryptophan-degrading, interferon-inducible enzyme that may down-regulate T cells by affecting local tryptophan catabolism, inhibiting anti-tumor immune mechanisms, and that has recently been shown to be involved in tumor resistance in a variety of hematopoietic malignancies. Another negative regulator of T-cell activation that was found among the most significantly up-regulated genes is VSIG4 (V-set and Ig-domain-containing 4, also known as Z39Ig). Thus both genes may contribute to immune suppression and a tolerogenic status. Not surprisingly, some of the signature data collected from THRLBCL overlapped with those of other unfavorable prognostic groups identified in follicular lymphomas[39] or diffuse large B-cell lymphomas.[35] An additional interesting finding was the lack of a T-cell signature in both NLPHL and THRLBCL, suggesting that both lymphomas have acquired several mechanisms to escape T-cell-mediated immune surveillance. The authors proposed that THRLBCL has a distinctive tolerogenic host immune response that is not

observed in NLPHL, and is mediated by macrophages and dendritic cells.

One concern about the data reported is that these patterns may just reflect a selection bias of the study design by analyzing only NLPHL cases with a predominant normal B-cell component (pattern A according to Fan *et al.*).[25] In such cases the lymphocyte-predominant cells are largely distributed in expanded follicles, containing a rich complement of IgD-positive mantle cells and usually some residual normal germinal center cells. The authors did not include NLPHL cases rich in T cells or cases with transitional features between NLPHL and THRLBCL.[24,25] However, such cases reflect an important part of the biology and natural history of NLPHL and their analysis could have provided additional biological clues to unravel similarities and dissimilarities between the two entities, NLPHL and THRLBCL.

Dr. Stefania Pittaluga and Dr. Elaine Jaffe are affiliated with the Hematopathology Section, Laboratory of Pathology, Center for Cancer Research, National Cancer Institute, National Institutes of Health. Dr. Pittaluaga is an Attending Staff Pathologist and Dr. Jaffe is Chief of the Hematopathology Section.

No potential conflicts of interests relevant to this article were reported.

References

1. Ramsay AD, Smith WJ, Isaacson PG. T-cell-rich B-cell lymphoma. Am J Surg Pathol. 1988;12(6):433-43.
2. Ng CS, Chan JK, Hui PK, Lau WH. Large B-cell lymphomas with a high content of reactive T cells. Hum Pathol. 1989;20(12):1145-54.
3. Macon WR, Williams ME, Greer JP, Stein RS, Collins RD, Cousar JB. T-cell-rich B-cell lymphomas. A clinicopathologic study of 19 cases. Am J Surg Pathol. 1992;16(4):351-63.
4. Arnold A, Cossman J, Bakhshi A, Jaffe ES, Waldmann TA, Korsmeyer SJ. Immunoglobulin-gene rearrangements as unique clonal markers in human lymphoid neoplasms. N Engl J Med. 1983;309(26):1593-9.
5. Macon WR, Cousar JB, Waldron JA, Jr., Hsu SM. Interleukin-4 may contribute to the abundant T-cell reaction and paucity of neoplastic B cells in T-cell-rich B-cell lymphomas. Am J Pathol. 1992;141(5): 1031-6.
6. Felgar RE, Steward KR, Cousar JB, Macon WR. T-cell-rich large-B-cell lymphomas contain non-activated CD8+ cytolytic T cells, show increased tumor cell apoptosis, and have lower Bcl-2 expression than diffuse large-B-cell lymphomas. Am J Pathol. 1998;153(6):1707-15.
7. Delabie J, Vandenberghe E, Kennes C, Verhoef G, Foschini MP, Stul M, et al. Histiocyte-rich B-cell lymphoma. A distinct clinicopathologic entity possibly related to lymphocyte predominant Hodgkin's disease, paragranuloma subtype. Am J Surg Pathol. 1992;16(1):37-48.
8. Chittal SM, Brousset P, Voigt JJ, Delsol G. Large B-cell lymphoma rich in T-cells and simulating Hodgkin's disease. Histopathology. 1991;19(3):211-20.
9. Fraga M, Sanchez-Verde L, Forteza J, Garcia-Rivero A, Piris MA. T-cell/histiocyte-rich large B-cell lymphoma is a disseminated aggressive neoplasm: differential diagnosis from Hodgkin's lymphoma. Histopathology. 2002;41(3):216-29.
10. Achten R, Verhoef G, Vanuytsel L, De Wolf-Peeters C. T-cell/histiocyte-rich large B-cell lymphoma: a distinct clinicopathologic entity. J Clin Oncol. 2002;20(5):1269-77.
11. Greer JP, Macon WR, Lamar RE, Wolff SN, Stein RS, Flexner JM, et al. T-cell-rich B-cell lymphomas: diagnosis and response to therapy of 44 patients. J Clin Oncol. 1995;13(7):1742-50.
12. Rodriguez J, Pugh WC, Cabanillas F. T-cell-rich B-cell lymphoma. Blood. 1993;82(5):1586-9.
13. Jaffe ES, Harris NL, Stein H, Vardiman J. Pathology and Genetics of Tumours of Haematopoietic and Lymphoid Tissues. Lyon, France: IARC Press 2001.
14. Rudiger T, Gascoyne RD, Jaffe ES, de Jong D, Delabie J, De Wolf-Peeters C, et al. Workshop on the relationship between nodular lymphocyte predominant Hodgkin's lymphoma and T cell/histiocyte-rich B cell lymphoma. Ann Oncol. 2002;13 (Suppl 1):44-51.
15. Achten R, Verhoef G, Vanuytsel L, De Wolf-Peeters C. Histiocyte-rich, T-cell-rich B-cell lymphoma: a distinct diffuse large B-cell lymphoma subtype showing characteristic morphologic and immunophenotypic features. Histopathology. 2002;40(1):31-45.
16. Lim MS, Beaty M, Sorbara L, Cheng RZ, Pittaluga S, Raffeld M, et al. T-cell/histiocyte-rich large B-cell lymphoma: a heterogeneous entity with derivation from germinal center B cells. Am J Surg Pathol. 2002; 26(11):1458-66.
17. Brauninger A, Kuppers R, Spieker T, Siebert R, Strickler JG, Schlegelberger B, et al. Molecular analysis of single B cells from T-cell-rich B-cell lymphoma shows the derivation of the tumor cells from mutating germinal center B cells and exemplifies means by which immunoglobulin genes are modified in germinal center B cells. Blood. 1999;93(8):2679-87.
18. Torlakovic E, Tierens A, Dang HD, Delabie J. The transcription factor PU.1, necessary for B-cell development is expressed in lymphocyte predominance, but not classical Hodgkin's disease. Am J Pathol. 2001;159(5):1807-14.
19. Marafioti T, Mancini C, Ascani S, Sabattini E, Zinzani PL, Pozzobon M, et al. Leukocyte-specific phosphoprotein-1 and PU.1: two useful markers for distinguishing T-cell-rich B-cell lymphoma from lymphocyte-predominant Hodgkin's disease. Haematologica. 2004;89(8): 957-64.
20. Loddenkemper C, Anagnostopoulos I, Hummel M, Johrens-Leder K, Foss HD, Jundt F, et al. Differential Emu enhancer activity and expression of BOB.1/OBF.1, Oct2, PU.1, and immunoglobulin in reactive B-cell populations, B-cell non-Hodgkin lymphomas, and Hodgkin lymphomas. J Pathol. 2004;202(1):60-9.
21. Kamel OW, Gelb AB, Shibuya RB, Warnke RA. Leu 7 (CD57) reactivity distinguishes nodular lymphocyte predominance Hodgkin's disease from nodular sclerosing Hodgkin's disease, T-cell-rich B-cell lymphoma and follicular lymphoma. Am J Pathol. 1993;142(2):541-6.
22. Kraus MD, Haley J. Lymphocyte predominance Hodgkin's disease: the use of bcl-6 and CD57 in diagnosis and differential diagnosis. Am J Surg Pathol. 2000;24(8):1068-78.
23. Nam-Cha SH, Roncador G, Sanchez-Verde L, Montes-Moreno S, Acevedo A, Dominguez-Franjo P, et al. PD-1, a follicular T-cell marker useful for recognizing nodular lymphocyte-predominant Hodgkin lymphoma. Am J Surg Pathol. 2008;32(8):1252-7.
24. Boudova L, Torlakovic E, Delabie J, Reimer P, Pfistner B, Wiedenmann S, et al. Nodular lymphocyte-predominant Hodgkin lymphoma with nodules resembling T-cell/histiocyte-rich B-cell lymphoma: differential diagnosis between nodular lymphocyte-predominant Hodgkin lymphoma and T-cell/histiocyte-rich B-cell lymphoma. Blood. 2003; 102(10):3753-8.
25. Fan Z, Natkunam Y, Bair E, Tibshirani R, Warnke RA. Characterization of variant patterns of nodular lymphocyte predominant Hodgkin lymphoma with immunohistologic and clinical correlation. Am J Surg Pathol. 2003;27(10):1346-56.
26. Swerdlow SH, Campo E, Harris NL, Jaffe ES, Pileri SA, Stein H, et al. WHO classification of Tumours of Haematopoietic and Lymphoid Tissues. 4th ed. Lyon, France: International Agency for Research on Cancer 2008.
27. Braeuninger A, Kuppers R, Strickler JG, Wacker HH, Rajewsky K, Hansmann ML. Hodgkin and Reed-Sternberg cells in lymphocyte predominant Hodgkin disease represent clonal populations of germinal center-derived tumor B cells. Proc Natl Acad Sci USA. 1997;94(17): 9337-42.
28. Marafioti T, Hummel M, Anagnostopoulos I, Foss HD, Falini B, Delsol G, et al. Origin of nodular lymphocyte-predominant Hodgkin's disease from a clonal expansion of highly mutated germinal-center B cells. N Engl J Med. 1997;337(7):453-8.
29. Ohno T, Stribley JA, Wu G, Hinrichs SH, Weisenburger DD, Chan WC. Clonality in nodular lymphocyte-predominant Hodgkin's disease. N Engl J Med. 1997;337(7):459-65.
30. Wlodarska I, Nooyen P, Maes B, Martin-Subero JI, Siebert R, Pauwels P, et al. Frequent occurrence of BCL6 rearrangements in nodular lymphocyte predominance Hodgkin lymphoma but not in classical Hodgkin lymphoma. Blood. 2003;101(2):706-10.
31. Franke S, Wlodarska I, Maes B, Vandenberghe P, Delabie J, Hagemeijer A, et al. Lymphocyte predominance Hodgkin disease is characterized by recurrent genomic imbalances. Blood. 2001;97(6): 1845-53.
32. Franke S, Wlodarska I, Maes B, Vandenberghe P, Achten R, Hagemeijer A, et al. Comparative genomic hybridization pattern distinguishes T-cell/histiocyte-rich B-cell lymphoma from nodular lymphocyte predominance Hodgkin's lymphoma. Am J Pathol. 2002; 161(5):1861-7.
33. Brune V, Tiacci E, Pfeil I, Doring C, Eckerle S, van Noesel CJ, et al. Origin and pathogenesis of nodular lymphocyte-predominant Hodgkin lymphoma as revealed by global gene expression analysis. J Exp Med. 2008;205(10):2251-68.
34. Schumacher MA, Schmitz R, Brune V, Tiacci E, Doring C, Hansmann

ML, et al. Mutations in the genes coding for the NF-{kappa}B regulating factors IκBα and A20 are uncommon in nodular lymphocyte-predominant Hodgkin lymphoma. Haematologica. 2010; 95(1):153-7.
35. Monti S, Savage KJ, Kutok JL, Feuerhake F, Kurtin P, Mihm M, et al. Molecular profiling of diffuse large B-cell lymphoma identifies robust subtypes including one characterized by host inflammatory response. Blood. 2005;105(5):1851-61.
36. Lenz G, Wright G, Dave SS, Xiao W, Powell J, Zhao H, et al. Stromal gene signatures in large-B-cell lymphomas. N Engl J Med. 2008; 359(22):2313-23.
37. Chetaille B, Bertucci F, Finetti P, Esterni B, Stamatoullas A, Picquenot JM, et al. Molecular profiling of classical Hodgkin lymphoma tissues uncovers variations in the tumor microenvironment and correlations with EBV infection and outcome. Blood. 2009;113(12):2765-3775.
38. Van Loo P, Tousseyn T, Vanhentenrijk V, Dierickx D, Malecka A, Vanden Bempt I, et al. T cell/histiocyte rich large B-cell lymphoma shows transcriptional features suggestive of a tolerogenic host immune response. Haematologica. 2010;95(3):440-8.
39. Dave SS, Wright G, Tan B, Rosenwald A, Gascoyne RD, Chan WC, et al. Prediction of survival in follicular lymphoma based on molecular features of tumor-infiltrating immune cells. N Engl J Med. 2004; 351(21):2159-69.

A novel subset of T-helper cells: follicular T-helper cells and their markers

Camille Laurent,[1] Nicolas Fazilleau[2] and Pierre Brousset[3]

[1]INSERM, U.563, Centre de Physiopathologie de Toulouse-Purpan, Toulouse, F-31300 France; [2]Université Paul-Sabatier, Toulouse, F-31400 France; [3]Laboratoire d'Anatomie Pathologique, CHU Purpan, Toulouse,France
E-mail: brousset.p@chu-toulouse.fr. *doi:10.3324/haematol.2009.019133*

(Related Original Article on page 432)

It has been known for a while that T-helper (T_H) cells are involved in the regulation of B-cell responses. The characterization of a precise subset of these specialized cells, called follicular T-helper cells (T_{FH}), was first reported in 2000.[1] T_{FH} cells are characterized by their homing capacity in CXCL13-rich areas, such as B-cell follicles, through CXCR5 expression and their ability to support immunoglobulin production. The differentiation and homing of T_{FH}, as well as their interactions with B cells, have been the subject of intense research and several markers have been identified as components of the T_{FH} signature. Thanks to gene expression profiling carried out on peripheral T-cell lymphomas, a T_{FH} signature has been reported in angioimmunoblastic T-cell lymphoma (AITL) and in follicular T-cell lymphoma.[2-4] Several markers that identify T_{FH} cells are now used in hematopathology to reinforce the diagnostic criteria of AITL. In this issue of the Journal, Marafioti *et al.*[5] describe that inducible T-cell co-stimulatory molecule (ICOS), a CD28 homolog implicated in the regulation of T-cell differentiation and function, appears to be a relevant marker for the diagnosis of T_{FH}-derived lymphoma such as AITL and follicular T-cell lymphoma. This review first summarizes the recent evidence on T_{FH} cell differentiation and the present state of knowledge on molecules expressed by T_{FH} cells. Then, from this perspective, the potential implications of T_{FH} cells in lymphomagenesis and as part of the tumor microenvironment are covered.

Lineage and differentiation of follicular T-helper cells

T_H cell responses are heterogeneous and effector function choice is imposed during initial activation of the naïve T_H cells through the expression of transcription factors. It is now clear that T_{FH} cells belong to a subset of T_H cells that differ from the other T_H cell subsets. Indeed, T_H1 cells express STAT4, STAT1 and T-box transcription factor/T-bet, T_H2 cells express GATA3, T_H17 cells express retinoid related orphan receptor (RORγt) and regulatory T cells (Treg) express FOXP3. It has recently been shown that the T_{FH} cell differentiation program is controlled by BCL6.[6,7] T_{FH} cells upregulate BCL6 which in turn blocks T_H1, T_H2 and T_H17 cell differentiation by repressing their selective transcription factors.[6] Moreover, BCL6 antagonizes the expression of Blimp-1 transcription factor, which is preferentially expressed by T_H cells in the T zone area.[6,8] BCL6 also represses the expression of miR-17-92, a micro RNA that down-regulates CXCR5 expression, which is essential for T_{FH} cell function. Finally over-expression of BCL6 in activated T_H cells induces expression of interleukin (IL)-6 receptor and IL-21 receptor, which are both required for T_{FH} cell generation as shown by the role of IL-6 and IL-21 in T_{FH} cell differentiation.[9] ICOS is also implicated in the regulation of T_{FH} cell differentiation. Once engaged with its ligand (ICOS-L), expressed on antigen-presenting cells including B cells, ICOS induces the production of helper cytokines such as IL-2, IL-4 and especially IL-10 and IL-21.[10] ICOS deficiency is associated with a reduction of germinal center formation and fewer T_{FH} cells, suggesting that ICOS signaling is essential in T_{FH} cell generation.[10] Recent reports described that T_{FH} cells can produce significant amounts of IL-4, interferon-γ and IL-17 which are normally associated with T_H2, T_H1 and T_H17 cells, respectively.[1,11] Thus, T_{FH} cells share phenotypic features with other T_H cell lineages raising the question of whether T_{FH} cells really do constitute a distinct lineage or whether they result from a conversion of effector T_H cells that acquire T_{FH} cell functions.

The development of follicular homing ability by activated T_H cells is the first event in the process of T_{FH} generation. Indeed, naïve T cells that express CD62L and CCR7 enter secondary lymphoid organs in the T-cell zones.[8,11] Up-regulation of CXCR5, the receptor for CXCL13, a chemokine produced by follicular dendritic cells which promotes B-cell entry into the follicle, occurs after the interaction between antigen-presenting cells and naïve T_H cells.[8] Thanks to the expression of CXCR5 and down-regulation of CCR7, T_{FH} cells move into the B follicle where they regulate antigen-specific B-cell responses via co-stimulatory signals delivered through CD28, OX40 and ICOS.[11]

Cell functions and markers of follicular T-helper cells

In addition to CXCR5, T_{FH} cells express markers such as CD25, CD69, CD95, CD57 (in humans only), OX40 (CD134) and CD40L (CD154) and induce over-expression of activation-induced cytidine deaminase in B cells. T_{FH}