The Life and Death of the Germinal Center: An Immunohistochemical Analysis

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Abstract

INTRODUCTION

The germinal center (GC) is one of the fundamental morphologic components of the normal lymph node. It is the morphologic expression of the immune system's antigen dependent cell response. There is a delicate interplay between T cells, dendritic/antigen presenting cells and B cells, culminating in the formation of antigen specific B cells.

In this study, we attempted to address the subtle immunohistochemical changes that occur in different physiologic stage of the germinal center as well as changes associated with pathologic states. We used a large array of immunohistochemical stains meant to address components of the B cell compartments, T cell compartments as well as histiocytic/dendritic cells.

MATERIALS AND METHODS

A series of cases was obtained from consult cases in our institution. Diagnoses were rendered based on morphologic and immunohistochemical findings. These cases were then analyzed by an extensive panel of immunohistochemical stains to look at different components of the lymph node and specifically the germinal centers. The panel included: CD3, CD20, CD10, BCL2, BCL6, CD21, CD23, CD35, FOXP1, GCET1, HGAL/GCET2, LMO2, MUM1, IgD, Ki67, PD1 and PD-L1. The following case types were evaluated: hyaline vascular Castleman disease, nodular lymphocyte predominant Hodgkin lymphoma (HL), lymphocyte-rich classic HL, angioimmunoblastic T cell lymphoma, follicular lymphoma, follicular hyperplasia (FH), florid FH, atypical FH (2), and primary follicles/paracortical hyperplasia.

RESULTS

In general, primary unreacted cells (primary follicle cells, AKA mantle cells) retained the same reactivity with IgD and all GC markers throughout different GC reactions. The GC cells varied with their intensity and distribution of stains in both physiologic and pathologic states. Staining for FDC markers (CD21, CD23, CD35) varied in the state of reactivity and pathologic states; in general CD21 was the most reactive throughout all compartments and physiologic states, with losses of CD23 and CD35 expression both in normal and neoplastic conditions. In general, reactive T follicular helper cells (PD1 reactive) were fairly constant in distribution and numbers in both physiologic and pathologic states. However, in some pathologic states, the degree of PD-L1 macrophages (or in rare cases plasmacytoid dendritic cells) were markedly increased.

CONCLUSIONS

Our study analyzed the findings of immunohistochemical staining in a variety of states of the germinal center reaction, both physiologic and pathologic. We found subtle differences in expression of follicular dendritic cell (FDC) markers across the range of cases, supporting the idea that these markers are expressed at different stages of activation of the FDC. Further, we found that expression of GC associated markers (CD10, BCL6, GCET1, HGAL/GCET2, LMO2) differs across different phases of the GC reaction, and vary in intensity in neoplastic reactions. Finally, we addressed the distribution of T follicular helper cells (PD1) and their relationship in reactive and neoplastic conditions with PD-L1 positive macrophages.

Background

The germinal center (GC) is one of the fundamental morphologic components of the normal lymph node. It is the morphologic expression of the immune systems antigen dependent cell response. There is a delicate interplay between T cells, dendritic/antigen presenting cells and B cells, culminating in the formation of antigen specific B cells.

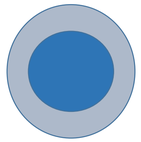
In this study, we attempted to address the subtle immunohistochemical changes that occur in different physiologic stage of the germinal center as well as changes associated with pathologic states. We used a large array of immunohistochemical stains meant to address components of the B cell compartments, T cell compartments as well as histiocytic/dendritic cells.

Results

PRIMARY FOLLICLE

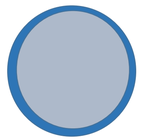
FDC	CD21 strong, dense networks. CD23 negative in FDC.
Lymph	HGAL (90%, weak), MUM1 (5%, strong), FOXP1 (90%, strong), IgD (90%, strong), Ki67 (5%, strong). Negative for CD10, BCL6, HGAL.
PD1/PD-L1	PD1 (5%, moderate). PD-L1 meshwork present (5-10%, moderate-strong)

REACTIVE FOLLICLE/FOLLICULAR HYPERPLASIA



FDC	CD21 strong in GC and mantle; CD23 strong in GC, negative in mantle
Lymph	CD10 (80%, moderate-strong), BCL6 (80%, moderate-strong), BCL6 (failed), HGAL (80%, strong), IgD negative, FOXP1 (10%, moderate), MUM1 (10% strong), Ki67 (90%, strong).
PD1/PD-L1	GC: PD1 (20%, strong). PD-L1 reticular network within GC and in interfollicular areas (moderate)

FLORID FOLLICULAR HYPERPLASIA



FDC	CD21 is strong and uniformly positive in FDC networks, but is only moderately strong in outer mantle zones. CD23 is strongly positive, but only in the most inner portions of the FDC network. CD35 is moderate-weakly positive in central portions of FDC networks.
Lymph	GC: CD10 and HGAL (90%, moderate-strong), BCL6 (90%, strong), GCET1 (90%, moderate) LMO2 (90%, strong), MUM1 (20%, moderate-strong) FOXP1 (40%, moderate-strong), Ki67 (95%, strong)
PD1/PD-L1	GC: PD1 (10%, strong). PD-L1 is positive in 5% of cells in germinal centers (moderate-strong). Mantle: There are rare/absent PD1 in mantle zones. PD-L1 is rare/absent in mantle zones.

MARGINAL ZONE HYPERPLASIA

FDC	No CD21/CD23 FDC networks in MZH; some CD23 staining in MZH cells
Lymph	FOXP1 (30%, moderate-strong), MUM1 and CD138 negative (no plasma cells), IgD (1-5%, weak), Ki67 (10-40%, moderate-strong)
PD1/PD-L1	PD1 negative; PD-L1 has dense strong staining in meshwork in MZH area

Results

ATYPICAL FOLLICULAR HYPERPLASIA

FDC	CD21 moderate-strong in dense networks. CD23 variable with strong staining at periphery, but usually weak in central portion.
Lymph	GC: CD10 (20-80%, moderate), BCL6 (40-80%, moderate-strong), GCET1 (10-20%, weak), HGAL (20-80%, strong), LMO2 (20-40%, weak), MUM1 (30%, strong), FOXP1 (negative), Ki67 (60-90%, strong), CD30 (variable, 90%, moderate-strong)
PD1/PD-L1	PD1 in germinal center (20%, strong). PD-L1 negative in GC and interfollicular areas.

CASTLEMAN DISEASE/FOLLICULAR ATRESIA

FDC	CD21 strong in former GC and mantle; CD23 strong in central portion; CD35 subset of CD21 but weak staining
Lymph	Mantle cells: IgD (90%, strong), BCL2 (90%, moderate-strong), LMO2 (90%, weak), FOXP1 (90%, strong); Negative CD10, BCL6, GCET1, HGAL or MUM1. Ki67 (<5%, strong)
PD1/PD-L1	PD1 rare to absent; PD-L1 negative in mantle zones

PROGRESSIVELY TRANSFORMED GERMINAL CENTER

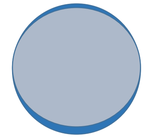
FDC	CD21 strong in large meshworks; CD23 shows minimal focal moderate-strong staining.
Lymph	Central: CD10 (80%, strong), BCL6 (80%, strong), GCET1 (40%, weak), HGAL (80%, strong), LMO2 (80%, strong), MUM1 (10%, strong), FOXP1 (80%, moderate), IgD (40%, weak), Ki67 (90%, strong) Mantle: LMO2 (80%, weak), FOXP1 (90%, weak), IgD (90%, moderate-strong), CD23 (40%, weak), Ki67 (10%, strong). Negative for CD10, BCL6, GCET1, MUM1
PD1/PD-L1	PD1 (20% weak); PD-L1 meshworks (5%, moderate)

NODULAR LYMPHOCYTE PREDOMINANT HODGKIN LYMPHOMA

FDC	CD21 strong in GC and mantle; CD23 strong in GC, negative in mantle
Lymph	LP cells: BCL6, GCET1, HGAL, LMO2 (weak), FOXP1 and MUM1 (weak, variable). Negative for CD10. Ki67 (90-100%, moderate-strong) Nodule B cells: LMO2 (70%, weak), FOXP1 (70%, moderate), MUM1 (70%, strong). Negative for CD10, BCL6, GCET1. Ki67 (30%, strong), IgD (90%, moderate)
PD1/PD-L1	PD1 moderate in T cells (variable %); LP cells moderate-strong PD-L1, focally.

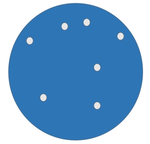
Results

FOLLICULAR LYMPHOMA



FDC	CD21 and CD35 strong dense staining in FDC; CD23 strong at periphery, weak in central portions
Lymph	GC: CD10/BCL6 (90%, moderate-strong), GCET1/HGAL (90%, weak). FOXP1 (1-10%, weak). MUM1 negative. IgD (90%, weak), Ki67 (5-20%, strong) mostly in large transformed cells
PD1/PD-L1	PD1 (5%, moderate-strong); PD-L1 rare in macrophages (<1%)

LYMPHOCYTE-RICH CLASSIC HODGKIN LYMPHOMA



FDC	CD21 is strongly positive in a diffuse meshwork in nodules; CD35 stains same pattern but moderate. CD23 stains only subset of dispersed networks strongly.
Lymph	Cells in nodules: CD10 (1%, weak), BCL6 (5%, weak), GCET1 (negative), HGAL (20%, weak), GCET1 (60%, weak), MUM1 (30%, moderate-strong), FOXP1 (70%, strong), IgD (strong, 30%). Ki67 (5%, strong) in nodules. Hodgkin cells: positive for BCL6 (weak), MUM1 (strong), FOXP1 (strong); negative for CD10, GCET1, HGAL, LMO2
PD1/PD-L1	PD1 expressed in scattered cells in nodules (20%, weak). PD-L1 is positive in Hodgkin cells (100%, strong); where Hodgkin cells are present, there are increased histiocytes which are positive in a meshwork (30%, moderate).

Observations

- FDC networks: CD21 stains meshworks robustly in all physiologic states and in most pathologic states. CD23 is upregulated in GC reaction and downregulated, and/or not expressed in primary follicles, in later GC reactions, and in many pathologic states
- Germinal center cells: The expression of GC-related markers (CD10, BCL6, GCET1 and LMO2) are fairly stable in physiologic but highly variable in pathologic states. Activation markers (FOXP1, MUM1) are highly variable in physiologic and pathologic states.
- Mantle type cells: These cells (including primary follicles, mantle zones, and cells of PTGC, LR-CHL and NLPHL) tend to have a relatively constant immunophenotype.
- PD1: T follicular helper cells tend to remain fairly constant in physiologic and pathologic states.
- PD-L1: The density and architecture of PD-L1 positive meshworks (histiocytes/accessory cells) are variable in different pathologic and physiologic states.