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Immunohistochemical Detection of γ/δ T-Lymphocytes in Formalin-Fixed Paraffin-Embedded Tissues

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Abstract

T-lymphocytes can be distinguished based on the composition of the T-Cell Receptor (TCR) chain in α/β T-cells and γ/δ T-cells. Correspondingly, α/β lymphomas can be distinguished from γ/δ lymphomas. The latter are rare neoplasms, which are usually confined to particular organs and tissues and carry a dismal prognosis. Until recently, monoclonal antibody (mAb) clone g3.20 to the TCR γ -chain was the reagent of choice for the immunohistochemical (IHC) detection of γ/δ T-cells and lymphomas in standard formalin-fixed paraffin-embedded tissues. Recently, due to technical problems, mAb g3.20 became recently unavailable. Our attempts to identify another commercially available clone to the TCR γ -chain were unsuccessful. However, we were able to identify a mAb (clone H-41, SC-100289, Santa Cruz, Dallas, TX) to the TCR δ -chain. H-41 works well in IHC on paraffin-embedded tissue and comparison with previously stained cases, shows superior immunolabeling to mAb g3.20. H-41 to the TCR δ -chain appears to be a suitable reagent for the replacement of mAb g3.20.

INTRODUCTION

Based on the composition of their heterodimeric T-cell receptor (TCR) chains, T-lymphocytes can be distinguished as α/β and γ/δ T-cells (Attaf, Legut, Cole, & Sewell, 2015). More than 90% of circulating T-cells express α/β chains, while approximately 10% display γ/δ chains. However, the latter are most abundant in epithelial tissues such as the gastrointestinal tract and skin where γ/δ T-cells form a much higher percentage and appear to be involved in the rejection of pathogens entering via the mucosal and cutaneous surfaces. Corresponding to the biology of the normal parental cells, neoplasms of γ/δ T-cells are rare and mainly occurring as special subtypes such as cutaneous or hepatosplenic lymphomas. Although a rare entity, γ/δ lymphomas display an aggressive biology with poor prognosis and need to be distinguished from α/β lymphomas (Belhadj et al., 2003; Foppoli & Ferreri,

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2015; Toro et al., 2003). Immunohistochemistry (IHC) for TCR chains is a common and reliable method to distinguish both entities. Until recently, immunohistochemical detection of γ/δ T-lymphocytes was exclusively based on monoclonal antibody (mAb) clone gamma3.20 (further referred to as g3.20), which was commercially available from various providers (TCR1153, Thermo-Fisher, Waltham, MA; NBP2-22487, Novus, Littleton, CO) to the TCR gamma chain (Garcia-Herrera et al., 2011; Rodriguez-Pinilla et al., 2013; Rouillet, Gheith, Mauger, Junkins-Hopkins, & Choi, 2009). Recently, this reagent became unavailable due to problems with the g3.20-producing hybridoma. Although the reagent was offered by several commercial providers, none of them was able to continue providing g3.20, indicating that all reagent preparations were generated by the same manufacturer. The reasons for the lack of a suitable reagent for accurate anti-TCR γ/δ analyses as well as the ensuing problems have been widely discussed among researchers such as the members of gamma-delta forum who are studying the biology of γ/δ T cells (<https://www.facebook.com/gammadeltaForum/>). In the present study, we describe the search for a serological reagent replacing clone g3.20 for the immunohistochemical identification of γ/δ T-cells in standard archival paraffin specimens, which rendered a commercially available mAb to the TCR δ chain as a suitable replacement.

MATERIAL AND METHODS

We tested various commercial reagents for the use in formalin-fixed paraffin-embedded archival tissue. Several anti-TCR γ reagents were tested:

Clone 5A6.E91 (Thermo-Fisher, TCR1061), clone 7A5 (TCR Vgamma9, Thermo-Fisher, TCR1720), clone B1 (Biolegend, San Diego, CA; # 331202). Faced with the problems in properly detecting the TCR γ/δ molecule, we widened our search for serological reagents reactive to the TCR δ chain.

We then identified anti-TCR δ clone H-41 (Santa Cruz Biotechnology, Dallas, TX; SC-100289) and employed a similar testing approach previously used for the analysis of anti-TCR γ reagents.

All assays were performed on a Leica Bond-3 (Leica, Buffalo Grove, IL) automated stainer platform. Initial analysis for all reagents was performed on normal tissues employing the previously developed CBMTB technology comprising lymphoid tissues as well as skin and gastro-intestinal mucosa (Frosina & Jungbluth, 2016). We then analyzed a limited set of γ/δ lymphomas previously tested positive with mAb g3.20 as well as a number of α/β lymphomas, which were previously immunopositive with mAb clone 8A3 to TCR β (BetaF1, Thermo-Fisher, TCR1151).

RESULTS

All reagents were tested in panels of normal and neoplastic tissue using the CBMTB and TMA method (Frosina & Jungbluth, 2016). The list of tested tissues comprised various normal organs such as tonsil, spleen, lung, lymph node, stomach, colon, small intestine, placenta, skin, skeletal muscle, neocortex, ovary, testis, uterus, thyroid, parotid gland, kidney

liver, and pancreas. Test protocols employed various antigen retrieval methods such as high and low pH buffers, enzyme digestion and combinations thereof.

All anti-TCR γ antibodies were tested at various dilutions (1:25 – 1:2K) and employing various antigen retrieval methods (high pH buffer, citrate buffer, enzyme digestion, no pretreatment). However, none of the tested anti-TCR γ serological reagents (Clone 5A6.E91, Thermo-Fisher, TCR1061; clone 7A5, Thermo Fisher, TCR1720; clone B1, Biolegend, # 331202) generated consistent and/or reproducible immunoreactivity indicative of the presence of γ/δ T-cell in the IHC analyses of any of the tested tissues. However, upon the first titration assay, H-41 displayed an immunoreactivity pattern indicative of γ/δ T-cells immunolabeling a subfraction of T-lymphocytes in the proper sites of lymphatic organs and the mucosa of the GI-tract. The protocol was further optimized and H-41 worked best at 1:150 (0.7 μ g/ml, 30 minutes primary incubation time) employing a high pH antigen EDTA retrieval solution (ER2; 30 minutes, Leica). We then tested a panel of 20 α/β lymphomas and 20 γ/δ lymphomas (10x cutaneous, 10x hepatosplenic) including 3 cases with bone marrow involvement. The γ/δ lymphoma cases were previously immunohistochemically identified with mAb g3.20 to the TCR γ/δ chain. H-41 was immunoreactive in all γ/δ lymphomas and no reactivity was seen in any of the tested α/β lymphomas. Moreover, H-41 showed very good staining in three (EDTA) decalcified bone marrow specimens and no modification of the protocol was necessary. Immunostaining was present in cutaneous as well as hepatosplenic γ/δ lymphomas. We also tested panels (five tissues each) of non-hematological tumors such as serous ovarian carcinomas, colorectal carcinomas, renal cell carcinomas, adeno and squamous cell carcinomas of the lung as well as metastatic melanoma. All were negative for H-41.

DISCUSSION

The sudden lack of a suitable serological reagent to detect γ/δ T-cells and neoplasms thereof poses a severe problem for accurate lymphoma diagnosis in surgical pathology. Our present data suggest that mAb H-41 to the TCR δ chain is a proper alternative to the previously employed standard reagent mAb g3.20, which until recently was in widespread use for the detection of γ/δ T-cells (Garcia-Herrera et al., 2011; Rouillet et al., 2009).

There are two major TCR δ chains, $\delta 1$ and $\delta 2$, which are associated with different biological functions of T-cells and different types of γ/δ lymphomas. Cutaneous γ/δ lymphomas are usually associated with the $\delta 2$ chain, while $\delta 1$ chains are seen in the hepatosplenic type (Tripodo et al., 2009). Since the manufacturer does not specify the epitope detected by mAb H-41, we were concerned about its potential specificity for one of the two TCR δ chains. However, immunoreactivity was present in hepato-splenic as well as cutaneous lymphomas indicating that mAb H-41 does not selectively react with one only one particular, i.e. $\delta 1$ or $\delta 2$ TCR chain but rather recognizes a consensus region present in both. We are aware that the biology of γ/δ T-cells and lymphomas thereof is complex. Various combinations of TCR chains can occur including combinations of the δ chain with α/β chains (Attaf et al., 2015). As a consequence, in a future extended analysis, a 100% match of the expression patterns of TCR γ versus TCR δ cannot be expected (Rodriguez-Pinilla et al., 2013). We are currently analyzing a larger series of cutaneous as well as hepato-splenic

lymphomas with mAb H-41 to elucidate the immunoreactivity of mAb H-41 in γ/δ as well as α/β lymphomas and to further delineate the presence of TCR chains in those types of lymphomas. We communicated our findings to various research groups, which have already employed H-41 as an anti- γ/δ TCR reagent in various applications. At present, no data indicating H-41 reactivity outside the γ/δ T-cell spectrum have been observed. Given the urgent need to identify a suitable reagent, we feel compelled to transmit our preliminary data indicating that H-41 is a suitable diagnostic reagent for the immunohistochemical detection of γ/δ T-cells in formalin-fixed paraffin-embedded tissue including decalcified bone marrow specimens.

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Figure 1.

Immunohistochemical staining of anti-TCR monoclonal antibody H-41 (Santa Cruz, Dallas, TX, SC-100289; Leica Bond-3 stainer platform) in a) normal tonsil, immunolabeling of a subfraction of interfollicular lymphocytes (obj. 10x), b) villus, small intestine, immunostaining of intraepithelial and lamina propria lymphocytes (obj. 40x), c) spleen with extensive infiltration of immunopositive lymphocytes in hepato-splenic γ/δ lymphoma (obj. 10x), and d) skin with extensive immunopositive lymphocytic infiltrates in cutaneous γ/δ lymphoma (obj. 10x), e) bone marrow biopsy with extensive involvement by γ/δ lymphoma (obj. 10x), high magnification of typical sinus involvement (obj. 60x).