Expression of Platelet-Associated Marker CD42b on Histoplasma

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Abstract

Background
In pathologic specimens, *Histoplasma capsulatum*, can be readily identified by morphology and special stains such as GMS or PAS. In most cases, it is rarely necessary to use more complicated techniques, although PCR and serologic methodologies exist.
Incidentally, while evaluating a bone marrow biopsy infected with histoplasmosis, we identified unusual staining using the platelet associated marker CD42b, also known as glycoprotein (GP) Ib, expressed on platelets and megakaryocytes. It is a robust immunohistochemical stain, allow easy identification of a megakaryocytes, megakaryoblasts and platelets. The unusual staining appeared to be on the surface of fungi, highlighting them from the background.
As a result, we evaluated multiple cases with histoplasmosis using CD42b staining to determine if there was immunohistochemical reaction in the fungal organisms.
M&M
13 cases were obtained from multiple institutions. Tissues were from lymph node (6), lung (3), soft tissue (3), and bone marrow (1). Original diagnoses of *Histoplasma* infection were confirmed by histologic evaluation and special stains (including PAS and GMS). Immunohistochemical staining for CD42b was performed in each case and compared to a GMS stain in the same tissue. In the index case, staining was also performed megakaryocyte/platelet markers, CD61 and Factor VIII.
RESULTS
We found a fairly high concordance between the results of a GMS stain and the CD42b staining in fungal organisms (9/13). In several cases, more organisms and larger numbers of possible organisms were identified using the CD42b stain. In three cases, staining for putative organisms was identified with no staining for GMS. In one case, no staining was identified on CD42b, but GMS positive organisms were in an area of tissue fall-off within a granuloma. In some cases, we found black or brown pigment (hemosiderin, lipofuscin) which caused confusing overlapping results on the GMS stain. In the CD42b stain, positive results were brown, and could be easily distinguished from the black pigments. No staining was identified in organisms for Factor VIII or CD61.
CONCLUSIONS
We found strong and reproducible staining in *Histoplasma* organisms using an immunohistochemical stain for CD42b, a platelet marker. Recent studies have shown that a distinctive fungal molecular, fusicoccin, a phytotoxic terpenoid, can interact with the GPIba, This interaction of fusicoccin stabilizes the platelet protein complex formed by GPIb-Factor IX and Factor V. While our study is only preliminary it may suggest that there is a molecular basis for the immunohistochemical findings reported. By implication, there are proteins present in fungi that may have significant interactions with platelet proteins. Finally, this interesting overlap could be diagnostically useful to identify cases of histoplasmosis with challenging morphologic findings.

Background

Diagnosis of fungal organisms using histopathology is often challenging. In most cases, organisms are not well-highlighted by standard H&E staining. In cases of concern, organisms are stained using PAS or GMS which allows highlighting of organisms by relying on specific characteristics of the fungal cell wall to improve visualization. While helpful, these techniques lack sensitivity and can be suboptimal as the carbohydrate coat of the organisms may be modified due to therapy or even individual variation among the organisms. As such, there are a variety of serologic studies that are used to provide confirmatory evidence of infection in patients. Immunoassays such as immunodiffusion, latex agglutination, Western blot and ELISA are used on serologic specimens to speciate fungal organisms.

Histoplasma species are seen most commonly in the Midwest and Southeast, as well as broad areas in South America. It is a dimorphic fungus and human infection is due to *Histoplasma capsulatum*. The yeast form is almost always encountered in human infection while the hyphal form is seen in soil.

In this study, we tested fungal organisms, primarily *Histoplasma*, using immunohistochemical staining for CD42b, a marker typically used for identification of megakaryocytes.

Materials and Methods

Cases were identified by known or suspected diagnoses of fungal infections from the files of Neogenomics (Aliso Viejo, CA; DPO), City of Hope National Medical Center (Duarte, CA; YSK), and Indiana University Medical Center (Indianapolis, IN; LC). All samples were obtained with respect to local standards for ethical research.

In each case, H&E and GMS stains were reviewed (DPO) to confirm the presence of fungal organisms. CD42b staining was performed on Ventana Benchmark (Tucson, AZ) using anti-CD42b antibody (Dako; Carpinteria, CA) using standard techniques.

Results

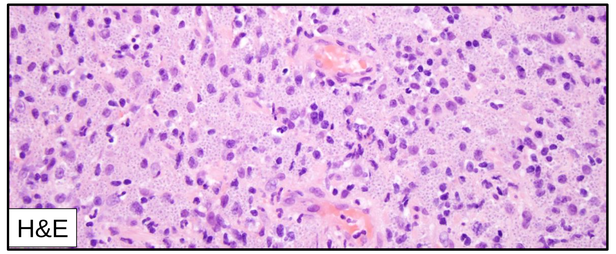
We identified an index case of histoplasmosis in a bone marrow biopsy of a 23 year male. The index patient had a history of living in the southwest United States, with recent U.S. military service in the Middle East one year prior. The patient had severe pancytopenia (indices: WBC 1.1 x10^3/uL; hemoglobin 7.4 g/dL; platelets 94 x10^3/uL) and splenomegaly. A peripheral blood smear did not reveal any organisms. A complete bone marrow evaluation was performed in the patient. In bone marrow aspirate smears, organisms were difficult to identify by Wright-Giemsa stain. They had an appearance and size comparable to platelets, and did not have a distinctive enough morphologic appearance to identify as fungal spores.

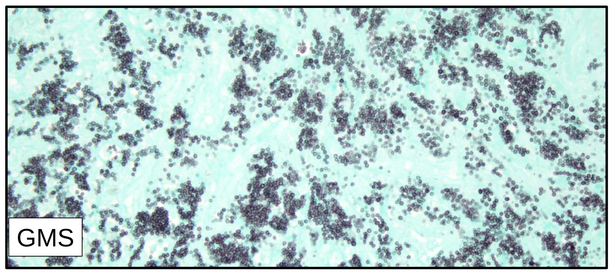
The bone marrow core biopsy was hypercellular for age (near 100%), but the overall hematopoietic marrow was reduced to approximately 20%, with macrophages comprising the majority of the bone marrow. The macrophages had bland, mature nuclei, and ample amounts of pale pink cytoplasm on H&E stain. Focally, the cytoplasm appeared slightly vacuolated, and rarely possibly small (2-3 micron) spherical structures could be appreciated.

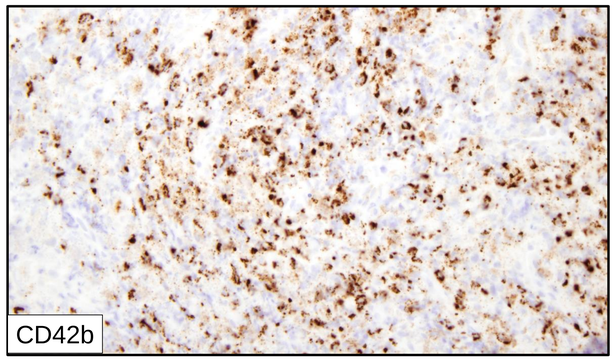
Because of the concern for infection AFB, PAS and GMS stains were performed. AFB was negative and both PAS and GMS stains revealed small (2-3 micron) round to oval organisms within the cytoplasm of macrophages. The staining by PAS was relatively weak, and staining for PAS and GMS were only seen in a minority of macrophages.

Immunohistochemical stains were also performed to evaluate the underlying marrow elements, as part of evaluation for other causes for the pancytopenia. CD34 and CD117 did not show any increase of immature myeloid elements or mast cells. CD3 stains increased scattered T cells associated with the increased macrophages.

In the index case, immunohistochemical staining for CD42b highlighted decreased numbers of megakaryocytes. It also highlighted numerous small organisms within the cytoplasm of most macrophages, with far more positivity than either GMS or PAS stain.







Results

A diagnosis of fungal infection, consistent with histoplasmosis was rendered. Subsequently, additional immunohistochemical staining for other megakaryocyte markers was performed; CD61 and Factor VIII antigen highlighted megakaryocytes but did not stain any organisms.

An additional 14 cases of previously diagnosed histoplasmosis were then stained with CD42b and GMS. In 11/14 cases, the GMS stain identified scattered organisms. Using staining for CD42b, organisms were identified in all cases (14/14) and were typically more numerous than those seen by GMS staining. CD61 was performed on two additional cases and was negative. Factor VIII was performed on one case and was negative.

Discussion

In most circumstances, infection with *Histoplasma* is asymptomatic, the majority of primary infections go unrecognized and only significant immunosuppression or severe infection lead to symptoms (Guimaraes 2006). Diagnosis typically requires a combination of clinical findings, serologic studies as well as histopathologic confirmation of organisms.

Our study supports that Histoplasma can be identified by CD42b immunohistochemical staining.

CD42b, also referred to as Gp1ba, is a platelet surface membrane glycoprotein. It binds with other platelet glycoproteins to bond to von Willebrand factor and cause platelet adhesion to surfaces.

We suggest the possibility that a fungal 14-3-3 protein, which are known to bond to Gp1ba (Zhang 2012), may share enough homology with the anti-CD42b (e.g. Gp1ba), to cause the staining effect that we have observed.

While this could present a potential pitfall in diagnosis, e.g. platelets misidentified as organisms and vice versa, we would suggest that it presents an interesting opportunity for both sensitive and relatively specific identification of pathogenic fungi in a variety of histopathologic samples. The presence of positivity for CD42b within macrophages with an appropriate morphology could lead to better identification of fugal organisms.

The histopathologic appearance of Histoplasma can be similar to other pathogens including *Candida, Penicillium, Pneumocytstis, Toxoplasma, Leishmania and Cryptococcus*. Testing of CD42b on a variety of other pathogenic fungal or other infections (such as *Candida*, *Aspergillus*, *Cryptococcus*, *Coccidioides*, *Blastomyces*, etc.), will provide insight into the range of reactive species. Cross reactivity may be present. However, even if a broad range of positive fungal organisms are identified, staining for CD42b would still allow for a sensitive immunohistochemical approach to identify fungi in histopathologic specimen.

References

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