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To our knowledge there are now reports on a total of 23 patients who have been studied for expression of the AML1/ETO transcript after allogeneic BMT.1-3 Of these patients, 15 have been found to have persistent expression, 7 were or became negative, and 1 was not evaluable. The discrepancy most likely reflects the lack of consensus on when to score a given sample as negative. Our data suggested that the sensitivity of each assay, the amount of starting material, or the source of the material (ie, BM v blood) might all contribute to some of the negative results obtained.3 For a patient to be considered negative at a given timepoint in our study, samples had to (1) be amplified in three independent experiments using 2.0 µg of total cellular RNA per reaction, (2) be successfully amplified for the β-actin in each reaction; (3) be performed simultaneously with an RT-PCR showing a sensitivity for detection of the AML1/ETO transcript of ≥1 × 106 in all three reactions, and (4) assays had to be performed on blood and BM. Taken together, these data suggest that, in the majority of patients, persistent expression of the AML1/ETO is compatible with continued clinical remission and, with the reported follow-up times of up to 10 years, even cure. Recently, similar results, although not after allogeneic BMT, were reported in childhood ALL.8

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Which Are the Nonerythroid Cells That Constitutively Express the Duffy Antigen?

To the Editor:

The Duffy blood group antigen has generated great interest because it is the receptor for the human malarial parasite Plasmodium vivax, simian malarial parasite Plasmodium knowlesi, and a new class of chemo kinase receptor for several proinflammatory cytokines.1-4 The finding that nonerythroid organs produce Duffy mRNA motivated the identification of cells that constitutively produce the Duffy protein (gp-Fy).5 Immuno histochemical studies were performed by Hadley et al,6 Peiper et al,7 and by Chaudhuri et al.8 Hadley et al and Peiper et al used only monoclonal antibody anti-Fy6. Chaudhuri et al used anti-Fy6 and rabbit polyclonal antibody 6615. The latter is a Duffy-specific antibody reacting with the sugar moiety of gp-Fy.4 According to Hadley et al and Peiper et al,7 endothelial cells of postcapillary venules of all organs and Purkinje cells of the cerebellum are the only nonerythroid cells that constitutively express gp-Fy. Chaudhuri et al8 identified the same cells; however, their studies showed gp-Fy in other cell types. Thus, in kidney, the endothelium of glomeruli, peritubular capillaries, vasa recta, and the principal cells (epithelial) of collecting ducts showed expression of gp-Fy. Duffy protein was also noticed in the endothelial cells of large venules and epithelial cells (type-I) of pulmonary alveoli. In thyroid, only the endothelial cells of capillaries produced gp-Fy. In spleen, in addition to the endothelial cells of capillaries and sinusoids, which is consistent with the observations of Peiper et al,7 endothelial cells of high endothelial venule (HEV) also produced abundant gp-Fy according to Chaudhuri et al.8 Furthermore, ultrastructural studies performed with antibody 6615 showed that apical and basolateral plasma membrane domains, including caveolae, contained gp-Fy. This indicates that the Duffy antigen is not limited to the membrane domain lining the vessels.

Hadley and Peiper8 challenged these findings in a recently published and well-documented review article. They disputed the specificity of rabbit polyclonal antibody 6615. However, Chaudhuri et al8 unequivo-
cally demonstrated 6615 specificity by performing the following experiments: (1) Immunoblots of membrane proteins from tissues and erythrocytes yielded the same reactive bands with either monoclonal antibody Fy6 or rabbit polyclonal antibody 6615; thus, both antibodies reacted with the same protein. (2) In the immunohistochemistry of the kidney, the cell types that reacted with monoclonal antibody Fy6 reacted also with antibody 6615. (3) Neither antibody reacted with stroma and parenchymal cells of adult liver, which is an organ that does not produce Duffy mRNA. (4) Antibody 6615, immunopurified by affinity chromatography, stained the same protein and the same cells as the nonimmunopurified 6615. The carbohydrate epitope that antibody 6615 recognizes is, therefore, only present in the Duffy protein. Despite these published results, Hadley and Peiper5 sustained that 6615 specificity remains to be determined. They incorrectly claimed that Chaudhuri et al6 only used antibody 6615. This is a misinterpretation of the data, because in kidney, the labeling of Duffy antigen throughout the entire collecting ducts was analyzed using anti-Fy6 as well as both nonimmunopurified and immunopurified 6615 antibodies. All of the antibodies stained the same cell types, but the labeling appears stronger in inner medulla and very weak in the cortex with monoclonal antibody anti-Fy6. Contrarily, Hadley et al6 restricted their studies to the cortex of the kidney using only anti-Fy6. This explains why the Duffy antigen was not observed in the principal cells of collecting ducts.7 In conclusion, the constitutive expression of Duffy antigen in the organs studied so far is noticed in certain epithelial cells, Purkinje cells of the cerebellum and endothelial cells of thyroid capillaries, post-capillary venules of some organs, and large pulmonary venules.

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The Duffy blood group antigen is a 7-membrane spanning protein that binds chemotactic cytokines (chemokines) from both C-X-C and C-C families.8,9 Immunohistochemical studies from our laboratory using a monoclonal antibody, anti-Fy6, showed that the Duffy chemokine receptor is present on endothelial cells of postcapillary venules, sinusoids of spleen, and Purkinje neurons of the cerebellum,8,9 Chaudhuri et al8 recently described a rabbit polyclonal antibody, designated 6615, that reacts with carbohydrate on the Duffy antigen. Immunohistochemistry with 6615 confirmed our results that the Duffy antigen is located on endothelial cells of postcapillary venules and sinusoids of spleen. In addition, reactivity with 6615 was noted with endothelial cells of other vascular structures, including renal glomeruli, capillaries, and vasa recta. Antibody 6615 also reacted with epithelial cells of collecting tubules in kidney and type I alveolar cells of lung.

In an addendum to a recent review on the Duffy antigen, we described the findings of Chaudhuri et al8 and stated “Whether or not the carbohydrate epitope recognized by polyclonal antibody 6615 is also present on molecules other than the Duffy antigen remains to be determined.” This comment was prompted by the Western immunoblots shown by Chaudhuri et al.8 In addition to the expected 35-43-kD Duffy reactive band (and expected higher molecular weight oligomers), the polyclonal rabbit antibody 6615 reacted with a lower molecular weight band of 21 kD seen with kidney and other tissues. A lower molecular weight band was also seen on immunoblots with anti-Fy6, but in the figure shown this band appeared higher than 21 kD. Also, the lower molecular weight band seen on immunoblots of kidney with 6615 was not seen with anti-Fy6 in the figure shown. The investigators stated that the lower molecular weight bands were probably degradation products of the Duffy antigen; however, this conclusion was not formally substantiated. In their Discussion, Chaudhuri et al8 characterized anti-Fy6 as giving an “unreliable signal in immunocytochemistry.” This has not been our experience. We have obtained consistent and strong reactivity with endothelial cells of postcapillary venules and Purkinje neurons of cerebellum using anti-Fy6. We have not seen reactivity with endothelial cells of large vessels such as arteries or veins except under conditions of inflammation (e.g., temporal arteritis, thrombophlebitis).9 Our data should be interpreted in light of the fact that we used a single (albeit reliable) monoclonal antibody, anti-Fy6. It has never been our contention that endothelial cells of postcapillary venules and Purkinje neurons of the cerebellum are the only cells that express Duffy. We simply described our findings using anti-Fy6 in immunohistochemical studies supported by Western immunoblotting and chemokine cross-linking experiments.8,9

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In summary, the immunohistochemical results reported by Chaudhuri et al. may indeed represent true expression of the Duffy chemokine receptor by the cells observed using antibody 6615. However, the possibility that the 21-kD 6615-reactive band represents a molecule other than a Duffy degradation product has not been formally excluded by the data presented. Both our studies and those of Chaudhuri et al. show that the Duffy chemokine receptor is expressed on vascular endothelial cells and other nonerythroid cells. The challenge now is to determine the function of this unique chemokine receptor. Insight into the function of the Duffy chemokine receptor will provide an important perspective from which to view the immunohistochemical data.

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Acquisition of Factor VIII Inhibitor After Acute Hepatitis C Virus Infection

To the Editor:

We report an unusual case of acquired serious factor VIII deficiency due to the development of human factor VIII inhibitor that occurred in an elderly female patient 2 months after having had an acute episode of hepatitis C virus (HCV). The case may suggest that the event could be a rare complication after HCV acute infection. An 84-year-old woman suffering from psoriasis, gastritis, and slight glucose intolerance was admitted to our division, in July 1996, because of jaundice and suspected acute hepatitis. The serum transaminase level was elevated (aspartate transaminase [AST] 923 U/L, alanine transferase [ALT] 765 U/L), showing hepatic cytosis, and the total bilirubin level was 13.9 mg%. An ultrasonographic examination of the liver showed the presence of a sclero-atrophic gallbladder, and biopsy of gastric mucosa, effectuated during gastroscopy, showed a chronic gastritis associated to Helicobacter pylori.

Abnormal ALT serum level (1,776 U/L) persisted during the first 2 weeks, subsiding thereafter gradually. The HCV antibody titer, which was determined by specific immune absorbent assays, increased from 0.55 to 1.57 optical density (OD) (normal range, 0.01 to 0.06) after 3 weeks stay at the hospital, after which she was discharged with diagnosis of HCV acute hepatitis. The recombinant immunoblotting assay showed antibody positivity against C-33 antigen; antinuclear autoantibodies, lupus anticoagulant, and ciprofloxin were negative. No bleeding or other signs of coagulopathy were reported at admittance until discharge: prothrombin time (PT) was 81%, partial thromboplastin time (PTT) was 55 seconds, and platelet count was 195,000/µL. Afterwards, the patient was observed at the divisional day hospital without evidence of complications. Two months later, the patient was readmitted with a large, spontaneous left arm hematoma, hematuria, and evidence of poor coagulation. The platelet count was 232,000/µL; PTT was prolonged to 93 seconds, PT was 75%, and only factor VIII, among other prothrombin factors, was found decreased to a level of 0.013 IU/mL (normal range, 0.70 to 1.50 IU/mL). The human factor VIII specific inhibitor was found to be positive (37.7 Bethesda unit [BU]/mL), whereas porcine factor inhibitor was 1.6 BU/mL. To avoid autoimmune response against porcine factor VIII, the patient was treated on activated prothrombin complex concentrate at 75 U/kg and methylprednisolone at 40 mg daily for 3 weeks, reserving treatment with porcine factor VIII concentrate for more severe events or high-risk situations of hemorrhagia.

No bleedings occurred any further and the patient was referred to a center for coagulation of another hospital. One month later, because of recurrence of hematuria, she underwent cystoscopic examination and a bladder benign papilloma was removed under coverage of preentive treatment with porcine factor VIII (100 U/Kg) everyday for 2 days. A week later, the patient was discharged.

A month later, the patient died at home after a severe acute bleeding episode of the gastrointestinal tract.

Development of inhibitors against factor VIII or factor IX is a common complication during specific substitutive treatment of hemophilia,1,2 and it is occasionally reported during interferon-a chronic therapy in patients with hemophilia A and chronic HCV hepatitis,3 but its relationship with acute or chronic HCV hepatitis is unknown.

The mechanism responsible for development of inhibitory reactivity against factor VIII in hemophilia A is related to the immunologic restricted specificity of inhibitor antibodies against regions on factor VIII protein; their reaction has been identified on immunoblotting.4

The 44-kD fragment from the heavy chain and the 72-kD fragment from the light chain of factor VIII, either or both, are immunogenic5 and increase antibody response toward them. Our patient did not have a history or signs of bleeding or spontaneous soft tissue hematomas before developing acute C hepatitis, and there has been no evidence of autoantibodies or clinical evidence of autoimmune diseases. This report suggests the possibility that, in this case, acute HCV infection could have induced the development of human factor VIII specific inhibitor.
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