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Rituximab in autoimmune cytopenias: for which patients?

The anti-CD20 monoclonal antibody rituximab represents a new tool for treating B-cell malignancies, especially low-grade lymphomas. More recently, the drug has been shown to be helpful in some patients with refractory autoimmune cytopenias, and the papers by Zaja *et al.*[1] and Delgado *et al.*[2] in this issue of Haematologica extend our knowledge on these new, emerging indications. The most convincing evidence of benefit from rituximab in this setting has been reported for patients with cold agglutinin disease. This uncommon hemolytic anemia, often associated with a chronic lymphoproliferative disease, is poorly responsive to steroids, cytotoxic chemotherapy or other therapeutic approaches. A favorable outcome, however, was observed in 12 of 14 patients (85%) given rituximab in a small prospective study[3] and in some case reports.[4-7] Three patients were previously untreated, whereas the others had failed several lines of conventional therapy. The results of rituximab treatment in patients with refractory warm-antibody autoimmune hemolytic anemia are less encouraging. Only a few case reports are available[8-10] and a complete hematologic remission has been achieved in about 50% of patients. In one study, anemia was reported to worsen after rituximab administration.[10] As far as concerns rituximab in chronic autoimmune thrombocytopenia, one cohort study on 25 patients has been published in full[11] and three other prospective series of more than 10 patients each have been reported in abstract or preliminary form.[12-14] Overall, a sustained increase of platelet count was achieved in 28% to 54% of patients resistant to previous treatments. Most investigators agreed that this drug should be carefully considered in severely thrombocytopenic patients who do not respond to standard therapy. The successful use of rituximab has also been reported in patients with pure red cell aplasia[9] and autoimmune thrombocytopenia and neutropenia,[15] refractory to conventional therapy.

The benefit of rituximab should be weighed against its potential risks. The drug is generally well tolerated, but a life-threatening syndrome of bronchospasm and/or hypotension has been described in about 10% of patients treated for lymphoma, particularly during the first infusion.[16] The syndrome is due to massive cytokine release from neoplastic cells and is more severe in patients with a high tumor burden and/or circulating tumor cells above 50,000/mL. Another side effect of the drug is the long-lasting impairment of humoral immunity, leading to an increased risk of viral and bacterial infections. Pure red cell aplasia due to parvovirus B19,[17] re-activation of acute hepatitis B[18] and bilateral bacterial pneumonia[19] have been reported after rituximab treatment. In occasional patients, the drug has also been associated with induction or exacerbation of autoimmune diseases, including acute agranulocytosis[20] and lupus thrombocytopenia.[21] (*Editorial note. The following paper on the use of rituximab recently appeared in this journal.*[22-29]).

Balancing benefits and risks, the possible indications for the use of rituximab in patients with autoimmune cytopenias are summarized in Table 1. Clearly, there is urgent need for further studies. The optimal schedule of administration of the drug and the duration of the clinical effect are important issues to be addressed. Most importantly, larger, prospective series of patients consecutively treated are required to avoid the bias of preferentially reporting successful cases. Ideally, all patients given rituximab for these and other still uncertain indications should be registered and evaluated. To

Table 1. Possible indications for the use of rituximab in autoimmune cytopenias.

- Patients with cold agglutinin disease, requiring treatment (ie. transfusion-dependent anemia and/or disease-related symptoms), also as first-line therapy (standard therapy usually ineffective)
- Patients with immune thrombocytopenic purpura, requiring treatment (ie. platelet count <10,000/mL and/or bleeding symptoms) and refractory to standard therapy
- Patients with warm-antibody autoimmune hemolytic anemia, pure red cell aplasia or immune thrombocytopenia and neutropenia, with severe disease requiring treatment and refractory to standard therapy*

*Standard therapy includes full-doses steroids, high-doses i.v. immunoglobulins, splenectomy and immunosuppressive drugs. *Evidence of benefit still limited in these indications. See text for details.*

reach this goal, a project of clinical epidemiology and surveillance of rituximab use has recently been launched and will be operative in all Italian hospitals very soon.

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References

1. Zaja F, Iacona I, Masolini P, Russo D, Sperotto A, Prosdocimo S, et al. B-cell depletion with Rituximab as treatment for immune hemolytic anemia and chronic thrombocytopenia. Haematologica 2002; 87:189-95.
2. Delgado J, Bustos JG, Jimenez-Yuste V, Hernandez-Navarro F. Anti-CD20 monoclonal therapy in refractory immune thrombocytopenic purpura. Haematologica 2002; 87:215-6.
3. Berentsen S, Tjonnfjord GE, Brudevold R, Gjertsen BT, Langholm R, Lokkevik E, et al. Favourable response to therapy with the anti-CD20 monoclonal antibody rituximab in primary chronic cold agglutinin disease. Br J Haematol 2001; 115:79-83.
4. Bauduer F. Rituximab: a very efficient therapy in cold agglutinins and refractory autoimmune haemolytic anaemia associated with CD-20 positive, low-grade lymphoma. Br J Haematol 2001; 112:1083-90.
5. Layios N, Van den Neste E, Jost E, Deneys V, Scheiff JM, Ferrant A. Remission of severe cold agglutinin disease after rituximab therapy. Leukemia 2001; 15:187-8.
6. Zaja F, Russo D, Fuga G, Michelutti T, Sperotto A, Fanin R, et al. Rituximab in a case of cold agglutinin disease. Br J Haematol 2001; 114:229-34.
7. Sparling TG, Andricevic M, Wass H. Remission of cold hemagglutinin disease induced after rituximab therapy. CMAJ 2001; 164:1405.
8. Ahrens N, Kingreen D, Seltsam A, Salama A. Treatment of refractory autoimmune haemolytic anaemia with anti-CD20 (rituximab). Br J Haematol 2001; 114: 244-5.
9. Zecca M, De Stefano P, Rotoli B, Locatelli F. Anti-CD20 monoclonal antibody for the treatment of severe, immune-mediated, pure red cell aplasia and hemolytic anemia. Blood 2001; 97:3995-7.
10. Grossi A, Santini V, Longo G, Balestri F, Rossi Ferrini P. Treatment with anti-CD20 antibodies of patients with autoimmune thrombocytopenia with or without haemolytic anemia: worsening in hemoglobin level. Blood 2000; 96:1089a [abstract].
11. Stasi R, Pagano A, Stipa E, Amadori S. Rituximab chimeric anti CD20 monoclonal antibody treatment for adults with chronic idiopathic thrombocytopenic purpura. Blood 2001; 98: 952-7.
12. Saleh MN, Gutheil J, Moore M, et al. A pilot study of the anti-CD20 monoclonal antibody rituximab in patients with refractory immune thrombocytopenia. Semin Oncol 2000; 27 Suppl 12:99-103.
13. Cooper N, Bussel J. Rituxan treatment in patients with immune thrombocytopenic purpura (ITP). Blood 2001; 98: 2180a [abstract].
14. Giagounidis AAN, Anhuf J, Schneider P, et al. Treatment of relapsed idiopathic thrombocytopenic purpura with the anti-CD20 antibody Rituximab. Blood 2001; 98: 2187a[abstract].
15. Faurschou M, Hasselbalch HC, Nielsen OJ. Sustained remission of platelet counts following monoclonal anti-CD20 antibody therapy in two cases of idiopathic thrombocytopenia and neutropenia. Eur J Haematol 2001; 66: 408-11.
16. Onrust SV, Lamb HM, Barman Balfour JA. Rituximab. Drugs 1999; 58:79-88.
17. Sharma VR, Fleming DR, Slone SP. Pure red cell aplasia due to parvovirus B19 in a patient treated with rituximab. Blood 2000; 96:1184-6.
18. Dervite I, Hober D, Morel P. Acute hepatitis B in a patient with antibodies to hepatis B surface who was receiving Rituximab. N Engl J Med 2001; 344:68-9.
19. Rosenthal E, Karsenti J-M, Pesce A, Cassuto J-L. Anti-CD20 monoclonal antibody (rituximab) administration in patients with refractory immunologic thrombocytopenic purpura. Blood 2001; 98:2194a[abstract].
20. Voog E, Brice P, Cartron J, et al. Acute agranulocytosis (AA) in three patients treated with Rituximab for non-Hodgkin lymphoma. Blood 2001; 98:4668a [abstract].
21. Mehta AC, Mtanos GJ, Gentile TC. Exacerbation of lupus while receiving Rituximab for chronic refractory thrombocytopenia. Blood 2001; 98:3862a [abstract].
22. Zaja F, Russo D, Fuga G, Patriarca F, Ermacora A, Baccarani M. Rituximab for the treatment of type II mixed cryoglobulinemia. Haematologica 1999; 84:1157-8.
23. Zinzani PL, Bendandi M, Gamberi B, Magagnoli M, Gherlinzoni F, Tura S. Complete molecular remission induced by rituximab in a patient with diffuse large cell lymphoma. Haematologica 2000 ; 85:437-8.
24. Coiffier B. Monoclonal antibodies in the treatment of non-Hodgkin's lymphoma patients. Haematologica 1999; 84 Suppl EHA-4:14-8.
25. Bermudez A, Marco F, Conde E, Mazo E, Recio M, Zubizarreta A. Fatal visceral varicella-zoster infection following rituximab and chemotherapy treatment in a patient with follicular lymphoma. Haematologica 2000; 85(8):894-5.
26. Dotti G, Rambaldi A, Fiocchi R, et al. Anti-CD20 antibody (rituximab) administration in patients with late occurring lymphomas after solid organ transplant. Haematologica 2001; 86:618-23.
27. Sacchi S, Federico M, Vitolo U, et al. Clinical activity and safety of combination immunotherapy with IFN-alpha 2a and Rituximab in patients with relapsed low grade non-Hodgkin's lymphoma. Haematologica 2001; 86:951-8.
28. Lauria F, Lenoci M, Annino L, et al. Efficacy of anti-CD20 monoclonal antibodies (Mabthera) in patients with progressed hairy cell leukemia. Haematologica 2000; 86: 1046-50.
29. Mazza P, Palazzo G, Amurri B, Pricolo G, Prudenzano A, Stani L. Rituximab with peripheral blood stem cell transplantation in CD20 lymphoproliferative disorders. Haematologica 2001; 86:1104-5.

Novel genes, proteins, and inherited disorders of iron overload: iron metabolism is less boring than thought

Until a few years ago, only three proteins of iron metabolism were known: transferrin, transferrin receptor and ferritin. From a clinical point of view, a step forward was the observation that both ferritin and transferrin receptor have soluble forms. The level of serum ferritin parallels the concentration of storage iron within the body, regardless of the cell type in which it is stored, so that determining serum ferritin levels represents a very convenient means of assessing iron balance in clinical practice.[1] The soluble transferrin receptor assay is much less employed in clinical settings, despite the fact that its determination is a convenient way of assessing erythroid activity.[2-4]

Of central importance to our understanding of iron metabolism was the later discovery that cellular iron homeostasis in mammalian cells is maintained by the co-ordinated regulation of transferrin receptor and ferritin synthesis that occurs at the translational level and is mediated by cytoplasmic mRNA-binding proteins, known as iron regulatory proteins (IRPs).[5] These proteins are capable of sensing cellular iron status and of interacting with mRNA stem-loop structures known as iron-responsive elements (IREs). Mutations that cause disease through increased efficiency of mRNA translation have been discovered, allowing recognition of hereditary hyperferritinemia/cataract syndrome and defining translational pathophysiology as a novel mechanism of human disease.[6]

Following the identification of HFE as the gene of HLA-related genetic hemocromatosis, homozygosity for the HFE C282Y mutation has been found in more than 90% of North European[7] and more than 80% of American patients of European origin clinically diagnosed as having genetic hemochromatosis.[8] However, markedly lower frequencies (50-64%) for C282Y homozygosity have been found in severely iron-loaded patients belonging to populations of Southern Europe.[9,10] Additional HFE mutations have been reported, the most frequent one being the A193T mutation leading to the S65C missense substitution,[11,12] while no conclusive evidence of abnormal gene expression in heterozygotes has been found.[13] The precise function of the HFE protein in physiologically inhibiting iron absorption[14] is not fully clear. The HLA-related hemochromatosis is defined as hemochromatosis HFE (OMIM 235200).

The availability of molecular tools for studying HFE mutations has allowed investigators to identify novel forms of non-HFE related genetic hemochromatosis (GH). Fifteen years ago we described juvenile GH as a distinct nosological entity.[15] Patients present with hypogonadotropic hypogonadism, and, unless proper treatment is started, die early because of cardiac dysfunction. In a collaborative study, the juvenile locus was later mapped to chromosome 1q.[16] Juvenile genetic hemochromatosis is defined as hemochromatosis type 2, or HFE2 (OMIM 602390). This gene must play a crucial role in iron metabolism, perhaps acting as the erythroid regulator of iron absorption hypothesized by Clement Finch decades ago.

In a few Italian families with non-HFE related hereditary hemochromatosis Camaschella *et al.* later found that affected individuals were homozygous for point mutations in the gene coding transferrin receptor 2 (TfR2), suggesting that this may represent the molecular basis for this familial iron overload syndrome.[17-19] TfR2 is a member of the *transferrin-like receptors* family with unknown function, but characterized by a restricted pattern of expression in the liver.[20] The TfR2 mutations responsible for hemochromatosis type 3, or HFE3 (OMIM 604250) appear to be private mutations.

In nearly simultaneous reports, a Dutch[21] and an Italian[22] group recently described pedigrees with atypical hemochromatosis inherited as an autosomal dominant trait. The two groups found different missense mutations in the gene encoding the iron export protein ferroportin1 (also known as IREG1, or MTP1).[23-25] This dominant type of genetic iron overload is defined as hemochromatosis type 4, or HFE4 (OMIM 606069). Ferroportin1 plays key roles in two different aspects of iron metabolism, absorption of dietary iron by duodenal enterocytes and release of iron from body stores by reticuloendothelial cells.

Nicolas *et al.*[26] recently reported severe tissue iron overload in upstream stimulatory factor 2 (USF2) knockout mice. Studies on genes that may account for the abnormalities of iron homeostasis in USF2(-/-) mice allowed the authors to identify hepcidin as the gene responsible for iron overload in these animals. This observation prompted Majore *et al.* to look for mutations in the hepcidin gene in Italian patients with non-HFE related GH. Their findings, reported in this issue,[27] are negative, but this does not exclude that hepcidin may be involved in the pathogenesis of inherited iron overload in humans, and that HFEn will be discovered in the near future.

Although the most common GH at present is the HFE-related disorder in population of Northern European extract, physicians working in other regions of the world are facing a less boring reality. Heterogeneity is challenging in both biology and clinical medicine, but it is also stimulating, inducing investigators to pursue new ideas and allowing science to progress.

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References

1. Finch CA, Bellotti V, Stray S, Lipschitz SA, Cool JD, Pippard MJ, Huebers HA. Plasma ferritin determination as a diagnostic tool. West J Med 1986;145:657-63.
2. Parisotto R, Gore CJ, Emslie KR, Michael J. Ashenden, Carlo Brugnara, Chris Howe, et al. A novel method utilising markers of altered erythropoiesis for the detection of recombinant human erythropoietin abuse in athletes. Haematologica 2000; 85:564-72.
3. Ho CH. The differential diagnostic values of serum transferrin receptor, serum ferritin and related parameters in the patients with various causes of anemia. Haematologica 2001; 86:206-7.
4. R'zik S, Loo M, Beguin Y. Reticulocyte transferrin receptor (TfR) expression and contribution to soluble TfR levels. Haematologica 2001; 86:244-51.
5. Klausner RD, Rouault TA, Harford JB. Regulating the fate of mRNA: the control of cellular iron metabolism. Cell 1993; 72:19-28.
6. Cazzola M, Skoda RC. Translational pathophysiology: a novel molecular mechanism of human disease. Blood 2000; 95:3280-8.
7. Jouanolle AM, Fergelot P, Gandon G, Yaouanq J, Le Gall JY, David V. A candidate gene for hemochromatosis: frequency of the C282Y and H63D mutations. Hum Genet 1997; 100:544-7.
8. Beutler E, Gelbart T, West C, Lee P, Adams M, Blackstone R, et al. Mutation analysis in hereditary hemochromatosis. Blood Cells Mol Dis 1996; 22:187-94.
9. Carella M, D'Ambrosio L, Totaro A, Grifa A, Valentino MA, Piperno A, et al. Mutation analysis of the HLA-H gene in Italian hemochromatosis patients. Am J Hum Genet 1997; 60:828-32.
10. Papanikolaou G, Politou M, Terpos E, Fourlemadis S, Sakellaropoulos N, Loukopoulos D. Hereditary hemochromatosis: HFE mutation analysis in Greeks reveals genetic heterogeneity. Blood Cells Mol Dis 2000; 26:163-8.
11. Remacha AF, Barcelo MJ, Sarda MP, Blesa I, Altes A, Baiget M. The S65C mutation in Spain. Implications for iron overload screening. Haematologica 2000; 5:1324-5.
12. Trombini P, Mauri V, Salvioni A, Corengia C, Arosio C, Piperno A. S65c frequency in Italian patients with hemochromatosis, porphyria cutanea tarda and chronic viral hepatitis with iron overload. Haematologica 2000; 86:316-7.
13. Vercesi E, Cerani P, Rolandi V, Rovati A, Bergamaschi G. Abnormal regulation of HFE mRNA expression does not contribute to primary iron overload. Haematologica 2000; 85:787-91.
14. Zuccon L, Corsi B, Levi S, Mattioli M, Fracanzani AL, Corti A, et al. Immunohistochemistry of HFE in the duodenum of C282Y homozygotes with antisera for recombinant HFE protein. Haematologica 2000; 85:346-51.
15. Cazzola M, Ascari E, Barosi G, Claudiani G, Dacco M, Kaltwasser JP, et al. Juvenile idiopathic haemochromatosis: a life-threatening disorder presenting as hypogonadotropic hypogonadism. Hum Genet 1983; 62:149-54.
16. Roetto A, Totaro A, Cazzola M, Cicilano M, Bosio S, D'Ascola G, et al. Juvenile hemochromatosis locus maps to chromosome 1q. Am J Hum Genet 1999; 64:1388-93.
17. Camaschella C, Roetto A, Cali A, De Gobbi M, Garozzo G, Carella M, et al. The gene TFR2 is mutated in a new type of haemochromatosis mapping to 7q22. Nat Genet 2000; 25:14-5.
18. Roetto A, Totaro A, Piperno A, Piga A, Longo F, Garozzo G, et al. New mutations inactivating transferrin receptor 2 in hemochromatosis type 3. Blood 2001; 97:2555-60.
19. De Gobbi M, Barilaro MR, Garozzo G, Sbaiz L, Alberti F, Camaschella C. TFR2 Y250X mutation in Italy. Br J Haematol 2001; 114:243-4.
20. Kawabata H, Yang R, Hirama T, Vuong PT, Kawano S, Gombart AF, et al. Molecular cloning of transferrin receptor 2. A new member of the transferrin receptor-like family. J Biol Chem 1999; 274:20826-32.
21. Njajou OT, Vaessen N, Joosse M, Berghuis B, van Dongen JW, Breuning MH, et al. A mutation in SLC11A3 is associated with autosomal dominant hemochromatosis. Nat Genet 2001; 28:213-4.
22. Montosi G, Donovan A, Totaro A, Garuti C, Pignatti E, Cassanelli S, et al. Autosomal-dominant hemochromatosis is associated with a mutation in the ferroportin (SLC11A3) gene. J Clin Invest 2001; 108:619-23.
23. Abboud S, Haile DJ. A novel mammalian iron-regulated protein involved in intracellular iron metabolism. J Biol Chem 2000; 275:19906-12.
24. McKie AT, Marciani P, Rolfs A, Brennan K, Wehr K, Barrow D, et al. A novel duodenal iron-regulated transporter, IREG1, implicated in the basolateral transfer of iron to the circulation. Mol Cell 2000; 5:299-309.
25. Donovan A, Brownlie A, Zhou Y, Shepard J, Pratt SJ, Moynihan J, et al. Positional cloning of zebrafish ferroportin1 identifies a conserved vertebrate iron exporter. Nature 2000; 403:776-81.
26. Nicolas G, Bennoun M, Devaux I, Beaumont C, Grandchamp B, Kahn A, et al. Lack of hepcidin gene expression and severe tissue iron overload in upstream stimulatory factor 2 (USF2) knockout mice. Proc Natl Acad Sci USA 2001; 98:8780-5.
27. Majore S, Binni F, Ricerca BM, Brioli G, Grammatico P. Absence of hepcidin gene mutations in 10 Italian patients with primary iron overload. Haematologica 2002; 87: 221-2.