the PETHEMA-LAL3/97 study. Haematologica 2003;88:445-53.
6. Spina M, Carbone A, Vaccher E, Gloghini A, Talamini R, Cinelli R, et al. Outcome in patients with non-Hodgkin lymphoma and with or without human immunodeficiency virus infection. Clin Infect Dis 2004;38:142-4.
7. Gerard L, Galicier L, Maillard A, Boulanger E, Quint L, Matheron S, et al. Systemic non-Hodgkin lymphoma in HIV-infected patients with effective suppression of HIV replication: persistent occurrence but improved survival. J Acquir Immune Defic Syndr 2002;30:478-84.
8. Matthews GV, Bower M, Mandalia S, Powles T, Nelson MR, Gazzard BG. Changes in acquired immunodeficiency syndrome-related lymphoma since the introduction of highly active antiretroviral therapy. Blood 2000;96:2730-4.
9. Levine A, Seneviratne L, Espina BM, Wohl AR, Tulpule A, Nathwani BN, et al. Evolving characteristics of AIDS-related lymphoma. Blood 2000;96:4084-90.
10. Tirelli U, Bernardi D. Impact of HAART on the clinical management of AIDS-related cancers. Eur J Cancer 2001;37:1320-4.

Multiple Myeloma

CD221 (IGF–1R) is aberrantly expressed in multiple myeloma, in relation to disease severity

We investigated the expression of insulin-like growth factor-1 receptor (CD221) in normal, reactive and malignant plasma cells. We show that CD221 is aberrantly expressed on human myeloma cells, that higher levels of CD221 are observed in patients and human myeloma cell lines with the most aggressive 14q32 translocations, and that CD221 expression has a negative prognostic impact in patients with multiple myeloma.

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Recent studies have shown that insulin-like growth factor-1 (IGF-1) is an important survival and growth factor in multiple myeloma (MM).[1-3] The studies of IGF-1 and interactions with its receptor IGF-1R or CD221 have mainly involved mouse models of plasmacytomas and/or human myeloma cell lines (HMCL)[4-6] but not MM patients, or normal plasma cells (PC). In this study we evaluated the expression of CD221 on normal, reactive and malignant PC, its correlations with presenting features of MM patients and its influence on the severity of the disease.

CD221 expression was evaluated on tumor cells from 56 consecutive patients with newly diagnosed MM, 10 extramedullary sites and 19 HMCL. Thirty-seven of these 56 MM patients (median age 60 years) treated in our center according to ongoing IFM protocols[7] (IFM99-02, 18 patients; IFM99-04, 9 patients; IFM 99-06, 10 patients) are included in the survival analysis. The 10 samples from extra-medullary sites were of malignant pleural effusions and peripheral blood from patients with plasma cell leukemias. The HMCL were either commercially available (L363, U266, OPM2, LP1, NCI H929, RPMI 8226), established by ourselves (XG1, XG2, XG6, Nan1, Nan2, Nan3, SBN, MDN, BCN) or generous gifts (KMS11, 12, 19, JIM3). Cells from tonsils, normal bone marrows, and reactive plasmacytoses were prepared as previously described.[8]

The phenotype of normal, reactive and malignant PC was analyzed in a four-color assay with anti-CD38, anti-CD45, and anti-CD138 monoclonal antibodies (mAb) as previously described by ourselves.[8-9] The CD of interest, CD221, was evaluated using the 1H7 mAb conjugated to phycoerythin (BD Biosciences) as previously described for CD20.[9] Positivity of CD221 expression was defined as a mean fluorescence intensity ratio MFIR ≥1.2. Fluorescent *in situ* hybridization (FISH) analysis of 13q and 14q32 abnormalities was performed on highly purified human myeloma cells as we previously described.[10] Non-parametric tests were used for statistical analysis. Usual presenting characteristics including cytogenetics and CD221 expression were included in the survival analysis. Survival curves were plotted according to the Kaplan-Meier method, and were compared using the log-rank test. CD221 was never detected on either tonsil or reactive PC, but was detected in 6 of 13 PC samples (46%) of normal bone marrow, at low levels (MFIR < 2) (p=0.05). Forty-one of the 56 patients (73%) expressed CD221. Levels of expression in MM were significantly higher than those of normal bone marrow (p< 0.05). Of note, CD221 was detected in 9 of 10 extramedullary sites, and in 17 of 19 HMCL. A strong correlation was found with 14q32 abnormalities and CD221 expression. HMCL with either t(4,14) or t(14,16) expressed CD221 at higher levels than did the remaining HMCL, including HMCL with t(11,14), those with non-recurrent 14q32 abnormalities and those lacking any 14q32 translocation (p=0.0049). Information on both –13q and 14q32 genotype and CD221 phenotype was available for the 56 patients. A non-significant link was found between CD221 expression and 13q deletion. On the other hand, a significant, strong correlation was found with 14q32 abnormalities. As in HMCL, CD221 was detected at higher levels in patients with either a t(4,14) or t(14,16) (p=0.0017). CD221 was expressed in 27 out of the 37 (73%) patients available for survival analysis. CD221 expression was associated with a shorter survival (median 35 months vs not reached, p<0.05, Figure 1). The other single factor associated with a shorter survival was t (4,14) (survival rate 53.6% vs 81.4% at 2 years, p<0.05). This is the first study comparing the expression of CD221/IGF-1R on normal, reactive and malignant PC. We did not detect any CD221 on 5 reactive plasmacytoses. Although the number of cases is small, our data clearly suggest that these expansions of highly proliferating but short-lived PC progenitors and precursors[7] do not use IGF-1 to survive and to grow. On the other hand, CD221 was detected (but at low levels) in 46% of normal bone marrow, but not in tonsil PC suggesting that CD221 could be specifically upregulated during the homing of PC in this special bone marrow microenvironment. In MM, we found that three-quarters of the patients expressed CD221 in agreement with a recent eval-

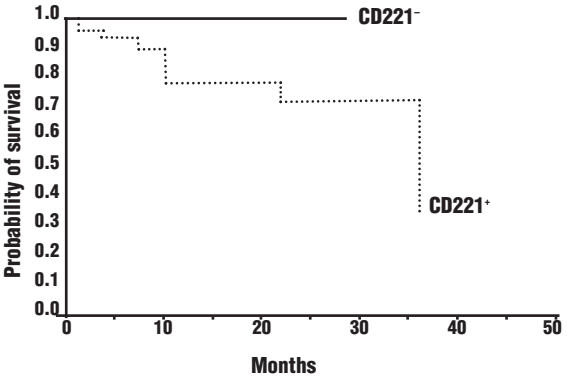

Figure 1. Overall survival according to CD221 phenotype. CD221 negative patients, n=10; CD221 positive patients, n=27; median survival: 35 months vs not reached, p<0.05).

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uation of 35 patients.[11] Results obtained from extra-medullary sites and HMCL, of which more than 85% of cases express CD221, always at high levels, suggest that CD221 expression could be upregulated during disease progression and associated with a more aggressive disease, and facilitates cell growth *in vitro*, in agreement with the biology of IGF-1 in mouse models.[4-5]

We found that CD221 expression was not random but correlated with t(4,14) and t(14,16) translocations, translocations generally associated with poorer prognosis in patients.[10] It also seemed that CD221 expression was related to disease severity, although given the small number of patients and their non-uniform treatment management, survival data should be interpreted cautiously. To conclude, the CD221 phenotype should be systematically evaluated in myeloma patients, and this receptor could be an ideal therapeutic target in patients, as recently shown.[11]

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References

1. Freund GG, Kulas DT, Mooney RA. Insulin and IGF-1 increase mitogenesis and glucose metabolism in the multiple myeloma cell line, RPMI8226. J Immunology 1993;151:1811-20.
2. Georgii-Hemming P, Jernberg Wiklund H, Ljunggren O, Nilsson K. Insulin-like growth factor I is a growth and survival factor in human multiple myeloma cell lines. Blood 1996;88: 2250-58.
3. Ferlin M, Noraz N, Hertogh C, Brochier J, Taylor N, Klein B. Insulin-like growth factor induces the survival of proliferation of myeloma cells through an interleukin-6-independent transduction pathway. Br J Haematol 2000;111:626-34.
4. Vanderkerken K, Asosingh K, Braet F, Van Riet I, Van Camp B. Insulin-like growth factor-1 act as a chemoattractant factor for 5T2 multiple myeloma cells. Blood 1999;93:235-41.
5. Ge NL, Rudikoff S. Insulin-like growth factor I is a dual effector of multiple myeloma cell growth. Blood 2000;96:2856-61.
6. Mitsiades CS, Mitsiades N, Poulaki V, Scholossman R, Akiyama M, Chauhan D, et al. Activation of NF-κB and upregulation of intracellular anti-apoptotic proteins via the IGF-1/Akt signaling in human multiple myeloma cells: therapeutic implications. Oncogene 2002;21:5673-83.
7. Harousseau JL, Shaughnessy J Jr, Richardson P. Multiple myeloma. Hematology 2004:237-56.
8. Jego G, Bataille R, Pellat-Deceunynck C. Interleukin-6 is a growth factor for non malignant human plasmablasts. Blood 2001;97:1817-22.
9. Robillard N, Avet-Loiseau H, Garand R, Moreau P, Pineau D, Rapp MJ, et al. The expression of CD20 is aberrant, significantly associated with a small mature plasma-cell morphology, t(11;14) and good survival in multiple myeloma. Blood 2003;102:1070-1.
10. Moreau P, Facon T, Leleu X, Morineau N, Huyghe P, Harousseau JL, et al. Recurrent 14q32 translocations determine the prognosis of multiple myeloma, especially in patients receiving intensive therapy. Blood 2002;100:1579-83.
11. Mitsiades CS, Mitsiades NS, McMullan CJ, Poulaki V, Shringarpure Akiyama M, Hideshima T, et al. Inhibition of the insulin-like growth factor receptor-1 tyrosine kinase activity as a therapeutic strategy for multiple myeloma, other hematologic malignancies, and solid tumors. Cancer Cell 2004;5:221-30.

Disorders of Hemostasis

The spectrum of mutations in Southern Spanish patients with hemophilia A and identification of 28 novel mutations

The aim of this study was to analyze the mutation pattern causing hemophilia A in a population from Southern Spain. Mutation analysis identified the mutation in 99 of the 109 unrelated patients enrolled in the Hemophilia Registry from Andalusia. About 54% of non-inversion mutations identified were previously unreported.

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Hemophilia A (HA) is an X-linked bleeding disorder caused by a wide spectrum of mutations in the coagulation factor VIII (*F8*) gene (MIM # 306700). In the severe phenotype, the most prevalent mutations are the intron 22 (IVS22) and intron 1 (IVS1) inversion accounting for 40-50% and 5% of the mutations, respectively.[1,2] Apart from these inversions, no mutation hotspots have been identified. Approximately 30% of all distinct point mutations in HA occur at CpG sites.[3]

The aim of this study was to analyze the mutation pattern causing HA in a population from Southern Spain. The study included a consecutive series of 109 unrelated males with HA (55 severe, 8 moderate and 46 mild phenotypes) enrolled in the Hemophilia Registry from Andalusia (Southern Spain). Forty were sporadic cases with no previous family history and 69 had a positive family history. Genealogical investigations conducted for each patient's family did not disclose any common ancestor in three generations.

Among the 55 patients with severe HA, of which 27 were sporadic cases (49%) and 28 had a positive family history (51%), the prevalence of IVS22[4] and IVS1[2]) inversion was 36% (20 patients) and 5% (3 patients), respectively. The IVS22 frequency was relatively low in comparison with the prevalence reported in other studies in the Spanish population.[1] Nevertheless, the number of patients in the group is too small to determine whether the frequency is statistically lower. When the correlation between familial or sporadic inheritance of the disease was analyzed, no significant differences were observed (11 and 9, respectively).

In patients with inversion-negative, severe or moderate-mild HA, we sequenced the *F8* gene (exons and intron/exon splice junctions) following standard protocols using previously described primers.[5] Among 32 patients with severe HA, the mutation was identified in 26 and 23 different mutations were found, 15 (65%) of which had not been previously reported;[6,7] none of these mutations affected the CpG sites. The novel mutations comprised 5 frameshift mutations, 4 nonsense mutations, 5 missense mutations and 1 mutation affecting the splicing sites (Table 1). Among 54 patients with moderate-mild HA, 12 sporadic cases (22%) and 42 with positive family history (78%), the mutation was identified in 50 patients and 29 different mutations were found (Table 2), all of them missense mutations. Thirteen (45%) of the 29 mutations identified were novel[6,7] and only one affected the CpG site. All the detected mutations were confirmed through two independent polymerase chain reaction

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