variant Ph translocation and this may account in part for the paucity of deletions.

Second, it is possible that a deletion is more likely to accompany a translocation with an M-bcr breakpoint. Since M-bcr breakpoints occur in the vast majority of patients with CML but in only a minority of patients with Ph-positive ALL, this could account for the rarity of deletions in the latter disease. Although the numbers are small our data are consistent with this idea since deletions were observed in only one of the 54 patients with an m-bcr breakpoint compared with 25 of 212 CML patients with a classical Ph translocation\(^{11}\) (\(P = 0.036\)).

Third, the rarity of deletions in Ph-positive ALL may reflect features of the target cell in which the translocation occurs. CML results from transformation of a multipotent stem cell,\(^3\) whereas ALL more often results from transformation of a committed B-cell progenitor.\(^{14}\) Lymphoid cells undergo antigen receptor rearrangements that require accurate joining of double stranded DNA breaks\(^{15}\) and may therefore employ mechanisms that minimize the concomitant occurrence of large deletions.

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References


To the editor:

Does the P\(_{2X1}\)del variant lacking 17 amino acids in its extracellular domain represent a relevant functional ion channel in platelets?

In a recent issue of Blood, Greco et al\(^1\) reported on the expression of a novel structurally altered P\(_{2X1}\) receptor in platelets and in megakaryocytic cell lines. This P\(_{2X1}\) variant lacks 17 amino acids in its extracellular domain due to a deletion within exon 6 of the P\(_{2X1}\) gene (GenBank accession no. 17481172). The authors showed that, after heterologous expression in the 321N1 astrocytoma cell line, P\(_{2X1}\)del subunits constitute a channel preferentially activated by adenosine diphosphate (ADP). In reverse transcriptase–polymerase chain reaction (RT-PCR) analyses, they described this variant as the major P\(_{2X1}\) mRNA of platelets, thus claiming that P\(_{2X1}\)del may play an important role as an ADP-activated ion channel in these cells. These conclusions are in contradiction with other studies\(^2,3\) that show that the functional platelet P\(_{2X1}\) receptor is an adenosine triphosphate (ATP)–gated ion channel that is unresponsive to high-performance liquid chromatography–purified ADP. Indeed, the activation of the P\(_{2X1}\) receptor by ATP or by its stable analogs, \(\alpha,\beta\)-methylene ATP and L-\(\beta,\gamma\)-methylene ATP, produces a rapid, quickly desensitized Ca\(^{2+}\) influx\(^4\) that is responsible for reversible platelet shape change,\(^1,5\) and that also plays a pivotal role during platelet aggregation induced by collagen.\(^3\) These platelet responses to ATP were found to be antagonized by ADP similarly to the inward current produced by ATP in Xenopus oocytes expressing wild-type P\(_{2X1}\) receptors (P\(_{2X1}\)wt).\(^6\) In addition, platelet receptors for ADP have been well characterized and are identified as 2 P\(_{2Y}\) receptors: P\(_{2Y1}\) and P\(_{2Y12}\) (reviewed in Gache\(^6\)). Both receptors are required for normal platelet responses to ADP, a conclusion recently corroborated in P\(_{2Y1}\)-/– and P\(_{2Y12}\)-/– knock-out mice.\(^7,8\) Thus, the existence in platelets of an ADP-activated variant of the P\(_{2X1}\) ion channel as the major platelet P\(_{2X}\) representative, as hypothesized by Greco et al.,\(^1\) can be questioned.

In this letter, we present data that argue against the possibility for a role of P\(_{2X1}\)del in platelet function. First, RT-PCR analyses of independent platelet RNA samples followed by sequencing of the PCR products showed major abundance of the P\(_{2X1}\)del mRNA (Figure 1A, lane 2; Figure 1B), whereas the platelet P\(_{2X}\)del mRNA appeared as a minor product. In contrast to the findings of Greco et al.,\(^1\) we found comparable relative amounts of the P\(_{2X}\)del mRNA in platelets and in the Dami megakaryocytic cell line (Figure 1A, lane 3; Figure 1B). Second, in order to further demonstrate the presence of P\(_{2X}\)del transcripts in platelets the authors designed 2 different...
sets of primers, S1/AS1 to amplify both P2X1del and P2X1wt cDNAs and S2/AS1 to amplify only P2X1del cDNAs. However, this strategy does not enable a quantitative assessment of P2X1del levels. Indeed, when the pcDNA3-P2X1del plasmid was used as a PCR template, we showed that the primer set S2/AS1 annealed to the P2X1del cDNA leading to artificial amplification of the 51 base pair deleted cDNA-encoding P2X1del (Figure 1A, lane 4, as confirmed by sequencing). This phenomenon evidently also can occur during RT-PCR analyses of platelet and Dami cell RNA samples containing both P2X1del and P2X1wt mRNA (Figure 1A, lane 5 and 6, respectively). Third, Western blotting experiments performed after transient transfection of 1321N1 cells with a pcDNA3 vector containing either the P2X1del cDNA (del) or the P2X1wt cDNA (wt). A nontransfected cell extract is also shown (NT). (C) Western blots of P2X1del and P2X1wt in transfected 1321N1 total cell extracts. The pcDNA3-P2X1del vector was transfected in 2 independent experiments (del 1 and del 2) and P2X1wt vector (wt). A nontransfected cell extract is also shown (NT). (D) Western blots of P2X1del (del) and P2X1wt (wt) proteins synthesized in a in vitro T7-coupled transcription/translation rabbit reticulocyte system. The rabbit polyclonal anti-human P2X1 antibody used in these experiments was previously described.9 Bands corresponding to P2X1del and P2X1wt PCR products and proteins are indicated. Molecular weight ladder is shown on the left.

Figure 1. Analyses of P2X1del mRNA and protein. (A) RT-PCR of platelet (lanes 2 and 5) and Dami cell (lanes 3 and 6) RNA; the primer sets S1/AS1 and S2/AS1 are described by Greco et al.1 In lanes 1 and 4, the pcDNA3-P2X1del plasmid was used as a PCR template. (B) Enlarged view of lanes 2 and 3. For these experiments, platelets were isolated from freshly drawn blood of at least 10 unrelated healthy volunteers. (C) Bands corresponding to P2X1del and P2X1wt PCR products and proteins are indicated. Molecular weight ladder is shown on the left.(

Response:

Functional adenosine diphosphate–activated P2X1del receptor

Oury et al have confirmed our recent identification of a P2X1del variant of the P2X1 receptor RNA in platelets and megakaryocytic DAMI cells.1 The complex array of nucleotide receptors they describe in different cell types suggests that questions of identity and function may not be fully resolved. What we have done, no more and no less, is to show that transfection of nonresponsive 1321 cells with a pcDNA3 vector containing either the P2X1del cDNA (del) or the P2X1wt cDNA (wt) revealed only low amounts of P2X1del proteins at the expected size in comparison to the P2X1wt protein levels (Figure 1C). To ensure that the antibody used in this detection would recognize the 2 proteins with equal sensitivity the P2X1del (del) and P2X1wt (wt) proteins were synthesized in an in vitro T7-coupled transcription/translation rabbit reticulocyte system. Western blotting analyses revealed identical amounts of the 2 in vitro–translated (nonglycosylated) proteins (Figure 1D). These data thus suggest that the P2X1del protein is not properly produced or is mainly unstable in the transfected 1321N1 cells.

Taken together, our data indicate that the P2X1del variant is unlikely to be a major protein in platelets. Moreover, the fact that Greco et al present this variant as a potential ADP-activated channel is not consistent with all the previous molecular and functional studies of platelet P2 receptors.6 The quantitative and functional relevance of the platelet P2X1del variant should therefore be reconsidered.3,4

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References

Role of surface IgM and IgD on survival of the cells from B-cell chronic lymphocytic leukemia

We read with great interest the paper by Bernal et al on the ability of anti–human IgM antibodies to prevent spontaneous apoptosis of B-cell chronic lymphocytic leukemia (B-CLL) cells in vitro. These findings suggest that receptor engagement by certain antigens, perhaps autoantigens that are constantly available in vivo, may contribute to the survival of the neoplastic cells.

The data, although interesting, are in contrast with previous findings of other groups including our own that showed that exposure to anti-μ antibodies causes rapid apoptosis of certain B-CLL cells. More recently we have also demonstrated that when an anti-Ig–δ chain antibody is used, prolonged cell survival instead of apoptosis is observed. From these findings it appears that the specificity of the antibodies to surface Ig plays a crucial role in the outcome of the experiments. Unfortunately, in the experiments by Bernal et al very little attention is paid to the specificity of the reagent used. If the anti-Ig reagent (a polyclonal goat anti-IgM antibody) that they used also reacted with light chains then the cells could have been stimulated via surface IgM and IgD. In these conditions, the prevailing physiological pathway would be survival or apoptosis depending on which of the 2 signals is more potent.

Differences of about 15% in the Annexin-V staining are barely significant with the presently used methods, according to the experience accumulated by several groups including our own. Thus, 3 B-CLL groups can be distinguished in the work of Bernal et al: those that do not respond at all to anti-IgM treatment (cases 31, 86, 89); those that barely respond (cases 47, 69, 72, 96, 104, 106, 108, 111, 114, 121); and those who definitely respond (cases 104, 106, 108, 111); and those who definitely respond (cases 31, 86, 89); those that barely respond (cases 47, 69, 72, 96, 104, 106, 108, 111, 114, 121); and those who definitely respond (cases 58, 78). The conclusions of the authors, as mentioned in the title of their article, are based primarily upon the latter 2 cases.

Bernal et al state that for our previous work we selected for cases expressing CD38. These cases might represent a special subset of B-CLL. CD38 expression distinguishes two groups of B-CLL cells: differential activation of the phosphatidylinositol-specific phospholipase C.

References

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