Does the P2X1del variant lacking 17 amino acids in its extracellular domain represent a relevant functional ion channel in platelets?

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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 translocation11 (P = 0.36).

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 rearrangements that require accurate joining of double stranded DNA breaks15 and may therefore employ mechanisms that minimize the concomitant occurrence of large deletions.

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References


To the editor:

Does the P2X1del 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 al1 reported on the expression of a novel structurally altered P2X1 receptor in platelets and in megakaryocytic cell lines. This P2X1 variant lacks 17 amino acids in its extracellular domain due to a deletion within exon 6 of the P2X1 gene (GenBank accession no. 17481172). The authors showed that, after heterologous expression in the 1321N1 astrocytoma cell line, P2X1del 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 P2X1 mRNA of platelets, thus claiming that P2X1del may play a role in platelet function.

These conclusions are in contradiction with other studies2,3 that show that the functional plateaue16 P2X1 receptor is an adenosine triphosphate (ATP)–gated ion channel that is unresponsive to high-performance liquid chromatography–purified ADP. Indeed, the activation of the P2X1 receptor by ATP or by its stable analogs, α,β-methylene ATP and L-β,γ-methylene ATP, produces a rapid, quickly desensitized Ca2+ influx that is responsible for reversible platelet shape change,4,5 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 P2X1 receptors (P2X1wt).6 In addition, platelet receptors for ADP have been well characterized and are identified as 2 P2Y receptors: P2Y1 and P2Y12 (reviewed in Gachet6). Both receptors are required for normal platelet responses to ADP, a conclusion recently corroborated in P2Y1–/– and P2Y12–/– knock-out mice.7,8 Thus, the existence of platelets of an ADP-activated variant of the P2X1 ion channel as the major P2X1 receptor, 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 P2X1del in platelet function. First, RT-PCR analyses of independent platelet RNA samples followed by sequencing of the PCR products showed major abundance of the P2X1del mRNA (Figure 1A, lane 2; Figure 1B), whereas the platelet P2X1del mRNA appeared as a minor product. In contrast to the findings of Greco et al,1 we found comparable relative amounts of the P2X1del 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 P2X1del transcripts in platelets the authors designed 2 different
with equal sensitivity the P$_{2X1del}$ (del) and P$_{2X1w}$ (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 P$_{2X1del}$ protein is not properly produced or is mainly unstable in the transfected 1321N1 cells.

Taken together, our data indicate that the P$_{2X1del}$ 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 P$_2$ receptors. The quantitative and functional relevance of the platelet P$_{2X1del}$ variant should therefore be reconsidered.

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References


Response:

Functional adenosine diphosphate–activated P$_{2X1del}$ receptor

Oury et al have confirmed our recent identification of a P$_{2X1del}$ variant of the P$_{2X1}$ receptor RNA in platelets and megakaryocytic DAMI cells. 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 the P$_{2X1del}$-variant cDNA results in the expression in these cells of a selective homomeric receptor sensitive to adenosine diphosphate (ADP).

Unfortunately, Oury et al have not provided adequate information for an evaluation of their polymerase chain reaction (PCR) results. We have previously shown that stringent annealing temperatures (60°C) are needed to minimize nonspecific binding due to the high degree of homology of the S2 and AS1 PCR primers. There are similar difficulties in evaluating their protein blots because their antibody appears to recognize multiple proteins in nontransfected cells, including the ADP receptor. In addition, Oury et al do not take into account the cross-reactivity of available antibodies for the P$_{2X1w}$ and P$_{2X1del}$ receptors. We do not feel that their conclusion that “the P$_{2X1del}$ protein is not properly produced or is mainly unstable in the transfected 1321N1 cells” can be deduced from these results.

Furthermore, contrary to the comments of Oury et al we have made no claims that “the P$_{2X1del}$ variant is a major protein in platelets.” In fact, data from the literature would suggest that the P$_2$ receptor would be of low abundance in platelets. However, our Ca$^{2+}$ influx studies clearly show that the P$_{2X1del}$ receptor is an ADP-activated channel, not a potential ADP-activated channel. Consideration of these problems, taken together with the extensive data reported in our original paper, shows that the question posed...
by Oury et al as the title of their communication must be answered in the affirmative.

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To the editor:

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 al1 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. 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 al1 (Table 1): 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 31, 86, 89). From these findings it appears that the cells from only about half of the B-CLL cases responded to anti-Ig stimulation in vitro as assessed in Ca2+ and plasma cell differentiation. 5 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.

In conclusion, the interesting hypothesis proposed by Bernal et al does not appear to be sustained by the available experimental data. It is possible that B-CLL cells are exposed to a variety of apoptotic and antiapoptotic signals in vivo and that the survival of a large fraction of them depends upon the fact that the antiapoptotic signals are prevailing.

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