Cross-talk of cGMP- and cAMP-signaling pathways in human platelets

Endothelium-derived prostacyclin and nitric oxide (NO) inhibit platelets by increasing the cytosolic concentrations of cAMP and cGMP, respectively. The increase of these two messengers leads to the activation of cAMP-dependent protein kinase (PKA) and cGMP-dependent protein kinase (PKG) and the subsequent phosphorylation of specific target proteins in platelets. One such protein is the vasodilator-stimulated phosphoprotein (VASP). VASP interacts with actin filaments and actin-binding proteins (zyxin, profilin) and is associated with focal adhesions in platelets. VASP is an in vitro substrate for both PKA and PKG, and within the 3 phosphorylation sites identified in VASP (Ser157, Ser239, Thr278), PKA has been reported to prefer the Ser157 site, and PKG the Ser239 site, suggesting that both enzymes might independently phosphorylate VASP in intact cells. Further support for a model of parallel and separate cAMP- and cGMP-signaling pathways in platelets came from a study of PKG-knockout mice demonstrating an absent cGMP-mediated VASP phosphorylation and platelet inhibition whereas the cAMP response was unchanged. Moreover, mice platelets lacking PKG showed an increased adhesion and aggregation during ischemia-reperfusion, indicating that an intact cGMP/PKG pathway was important to protect platelets in vivo (Massberg et al, J Exp Med. 1999;189:1255-1263).

The article by Li and colleagues (page 4423) shows surprisingly that the situation is over, mice platelets lacking PKG showed an increased adhesion and aggregation during ischemia-reperfusion, indicating that an intact cGMP/PKG pathway was important to protect platelets in vivo. Bernasconi and colleagues report here that TLR9 (the receptor required for recognition of CpG motifs) was low on naive B cells but high on memory B cells, and TLR9 mRNA was rapidly up-regulated in naive B cells upon ligation of the BCR. The authors propose a model in which the level of TLR9 expression modulates the type of B-cell response. Although this conclusion is tempting, no formal evidence is provided for such a causative relationship between these 2 phenomena (up-regulation of TLR9 and the B-cell differentiation). Not only memory but also naive B cells responded to CpG ODN in the absence of BCR ligation, although the naive B-cell response was limited to up-regulation of CD69 and CD86. Therefore, BCR ligation may trigger cellular events other than TLR9-up-regulation licensing naive B cells to proliferate and produce Ig in response to CpG. In this context it is interesting to note that in plasmacytoid dendritic cells (the other of the 2 CpG-sensitive immune cell subsets in humans), TLR9 expression is rapidly downregulated in the presence of the growth factor IL-3; still, CpG-mediated activation of plasmacytoid dendritic cells is even increased after preincubation with IL-3, suggesting that additional adaptor proteins modulated by IL-3 are involved in modulating the strength of the CpG-mediated response (Hornung et al, J Immunol. 2002;168:4531-4537).

As stated by Bernasconi and colleagues and evidenced by our own results, B cells are sensitive to CpG but not to LPS. The availability of CpG as the first defined microbial molecule recognized by human B cells leaves us with an avenue of new aspects of B-cell biology to be addressed, including B-cell malignancies (Jahrsdorfer et al, J Leukoc Biol. 2002;72:83-92).

CpG: unraveling the key to B-cell function

B cells were the first human immune cell subset known to recognize CpG motifs within microbial DNA in a CpG-specific manner. Based on B-cell activation and proliferation, the human CpG motif was identified and an oligonucleotide was developed (CpG ODN 2006) that induced the MAP kinase pathway and NFkB translocation in purified B cells, and that turned out to be a potent adjuvant to support humoral immune responses in primates (Hartmann and Krieg, J Immunol. 2000;164:944-953). But the important question of how CpG ODN acts on different levels of B-cell differentiation while maintaining antigen specificity remained elusive until recently, when Lanza-vaccia’s group reported that only memory B cells, and not naive B cells, proliferate and produce Ig in response to CpG ODN 2006 (Bernasconi et al, Science. 2002;298:2199-2202). In this issue, the same group (page 4500) extends these studies by demonstrating that naive B cells start to proliferate and to produce Ig in response to CpG ODN 2006 only if they receive simultaneous antigen-specific stimulation via the BCR. Synergy of BCR ligation and CpG ODN has been described before but so far has not been studied in the context of B-cell subsets and TLR expression. Bernasconi and colleagues report here that TLR9 (the receptor required for recognition of CpG motifs) was low on naive B cells but high on memory B cells, and TLR9 mRNA was rapidly up-regulated in naive B cells upon ligation of the BCR. The authors propose a model in which the level of TLR9 expression modulates the type of B-cell response. Although this conclusion is tempting, no formal evidence is provided for such a causative relationship between these 2 phenomena (up-regulation of TLR9 and the B-cell differentiation). Not only memory but also naive B cells responded to CpG ODN in the absence of BCR ligation, although the naive B-cell response was limited to up-regulation of CD69 and CD86. Therefore, BCR ligation may trigger cellular events other than TLR9-up-regulation licensing naive B cells to proliferate and produce Ig in response to CpG. In this context it is interesting to note that in plasmacytoid dendritic cells (the other of the 2 CpG-sensitive immune cell subsets in humans), TLR9 expression is rapidly downregulated in the presence of the growth factor IL-3; still, CpG-mediated activation of plasmacytoid dendritic cells is even increased after preincubation with IL-3, suggesting that additional adaptor proteins modulated by IL-3 are involved in modulating the strength of the CpG-mediated response (Hornung et al, J Immunol. 2002;168:4531-4537).

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Resistance to imatinib mesylate in CML: all BCR-ABL mutations “are created equal but some are more equal than others”

On May 2001, imatinib mesylate (Glivec, Gleevec) made the cover of Time as the magic bullet to cure cancer. In spite of the rather sensationalist and oversimplified style of the announcement, the drug did in reality represent a landmark for targeted therapy in neoplasia. The idea that a chemical compound could be designed and refined to fit into and block a specific domain of an oncprotein was in itself a vindication of the decades spent on research into the molecular biology of these proteins. The fact that the idea worked in practice, with the designer compound turning out to be an overwhelmingly successful drug, looked indeed like magic. For the treatment of chronic myeloid leukemia (CML), a new era had indeed begun with this selective inhibitor of the Bcr-Abl tyrosine kinase. Unfortunately, however, imatinib is somehow a victim of its own success: its target specificity and its “snug fit” into the Abl kinase pocket provide the ideal scenario for the leukemic population to evade its action, if some leukemia cells can produce Bcr-Abl molecules with mutant amino acids that directly or indirectly affect imatinib binding. As with conventional antibiotic resistance in bacteria, the mutant clone will be able to outgrow the wild-type sensitive cells, and so the leukemia will no longer respond to imatinib.

True to such a somber forecast, shortly after the encouraging results of the first clinical trials were announced, there came the first reports of resistance to imatinib occurring as a consequence of point mutations in the Bcr-Abl kinase domain. To date, at least 19 different mutations have been identified in cells from patients who became refractory to imatinib treatment (Goldman and Melo, N Engl J Med. 2003, in press). Since the original, preimatinib leukemia clone in all these cases comprised wild-type BCR-ABL–positive cells, the point mutations in the newly emerged resistant clone seem to be the obvious “cause” for the imatinib-resistant phenotype. But are they always? In this issue, Corbin and colleagues (page 4611) sound a warning note. They show that, whereas several mutants detected in relapsed patients have in fact a significantly lower in vitro sensitivity to imatinib's anti-proliferative and antiphosphorylation effects, others remain fully inhibited by the drug. In these cases, the resistant phenotype may be due to yet another abnormality, maybe another coexisting but unidentified point mutation that could even lie outside the kinase region (Azam et al, Cell. 2003;112: 831-843), or maybe an abnormality downstream of Bcr-Abl leading to a Bcr-Abl–independent mechanism of resistance.

The question of how the mutant cell clone is selected to become the predominant one at the time of relapse when the mutation itself does not confer a growth advantage in the presence of the drug remains to be explained. Equally intriguing is the opposite question: why are some of the mutations in amino acids predicted to be important and shown to be responsible for in vitro resistance (Azam et al) not actually seen in patients? And similarly, why are there some amino acid residues known to be in contact with imatinib that are never apparently mutated in either in vitro or in vivo resistance? Could it be that all these mutations would also result in the loss of kinase activity and transformation effect? These and other related issues tell us that Bcr-Abl still has a trick or two up its sleeve. Or in its pocket!

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Myocardial irony

Iron-induced heart disease remains the most frequent cause of death in thalassemia major and a critical life-limiting complication of other transfusion-dependent disorders, hereditary hemochromatosis, and other forms of iron overload. Cardiac iron toxicity is insidious, characteristically remaining clinically covert until heart failure and arrhythmias abruptly appear. Early detection of patients at increased risk by endomyocardial biopsy is ineffective because iron is deposited heterogeneously in the heart. As summarized in a recent NIDDK workshop (Brittenham and Badman, Blood. 2003;101: 15-19), magnetic resonance (MR) studies offer a potential noninvasive means of identifying patients with heart iron deposition. Use of MR to assess cardiac iron has been hindered because clinical MR instruments detect tissue iron indirectly by the magnetic effects of ferritin and hemosiderin iron on nearby hydrogen nuclei. Because a detailed theoretical understanding of these complex interactions is lacking, empirical efforts have used a variety of instruments, imaging sequences, and parameters.

In this issue, Jensen and colleagues (page 4632) report the first repeated MR measurements of cardiac iron in transfused patients treated with deferoxamine. Using signal intensity ratios, they found increased cardiac iron (with one exception) only in patients with a liver iron concentration above a threshold concentration (350 μmol/g dry weight), as well as significant correlations between heart iron and serum ferritin concentration and, intriguingly, deferoxamine-induced urinary iron excretion. As the authors emphasize, other MR approaches (T2, T2*, magnetization transfer ratios) have yielded seemingly contradictory results (eg, no hepatic iron threshold for cardiac iron, no significant relationship with serum ferritin). Jensen and his colleagues, painstaking pioneers in the use of MR for studies of tissue iron, caution that clinical use of MR estimates of myocardial iron must await an “ironing out” of these apparent anomalies.

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