

## TxA<sub>2</sub> Receptor-Based Vaccination: A Novel Potential Therapeutic Approach to Limit Thrombosis

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**A** aberrant intravascular platelet activation leads to thrombosis, which is the most common cause of ischemic heart attack and stroke. At the site of vascular injury, platelet activation and aggregation lead to the first wave of thrombin, a potent platelet activator, generation which helps in thrombus stabilization.<sup>1</sup> Apart from thrombin, activated platelets also release ADP and arachidonic acid as secondary mediators that potentiate platelet activation and aggregation. The platelet activation and aggregation cascade is initiated by a variety of platelet receptors including G protein-coupled and other transmembrane receptors, such as protease-activated receptors (PAR1/4), P<sub>2</sub>Y<sub>12</sub>, glycoprotein (GP) VI and Ib/IX/V, integrin αIIbβ<sub>3</sub>, and thromboxane A<sub>2</sub> (TxA<sub>2</sub>) receptor (TPR).<sup>2</sup> These receptors and other platelet-signaling molecules have been extensively targeted to identify the novel antithrombotic targets, and these efforts, in fact, led to the development of several small molecule inhibitors.<sup>3</sup> Of these, some are already being used in the clinic (eg, aspirin, clopidogrel) to prevent or limit the reoccurrence of thrombosis, and others are being tested for therapeutic efficacy and are at different levels of clinical trials. The major limitation of these antiplatelet agents involves the increased risk of bleeding in patients. Consequently, better and safer therapeutic strategies are needed to limit thrombotic and cardiovascular events.

In this issue of the *Journal of the American Heart Association (JAHA)*, Alshbool et al<sup>4</sup> reported excellent in vivo results for a peptide-based vaccine against TPR. Platelet TPR, a G-protein-coupled receptor, is clinically important because it mediates

platelet activation and aggregation through TxA<sub>2</sub> binding.<sup>5</sup> COX-1 (cyclooxygenase 1) stimulation in platelets leads to the conversion of arachidonic acid to TxA<sub>2</sub>. The TxA<sub>2</sub> pathway is targeted through aspirin, a widely used antithrombotic drug, although the effect of aspirin is often compromised by agonists such as isoprostanes.<sup>6</sup> Aspirin was found to be nonselective for TxA<sub>2</sub> because it also inhibits prostaglandin I<sub>2</sub>,<sup>7</sup> and an increased rate of aspirin resistance has also been identified worldwide.<sup>8</sup> The use of aspirin is effective, with some limitations; targeting TPR could be a better way to achieve complete inhibition of downstream signaling. Several drugs that either target thromboxane synthase or antagonize TPR have been developed with the hope of providing better and complete inhibition of the TxA<sub>2</sub> pathway.<sup>6</sup> These drugs hold promise, but none of the clinically proven TPR antagonists are available yet.

Alshbool et al<sup>4</sup> used a novel approach to inhibit TPR. They showed interesting results from a study testing a vaccine against platelet TPR. To achieve this, the authors immunized the mice through a peptide-based vaccination approach using the TPR C-EL2 (C-terminus of the second extracellular loop) peptide. Previously, these authors and other groups mapped the TPR ligand binding domain and found that it lies between C<sup>183</sup> and D<sup>193</sup> regions of C-EL2.<sup>9,10</sup> Taking these studies further, authors have previously characterized C-EL2Ab (antibody against C-EL2 domain) and showed that the antibody selectively blocks TPR-mediated platelet aggregation.<sup>11</sup> Along the same lines, they have characterized C-EL2Ab in vivo and concluded that the antibody protects mice against the development of occlusive arterial thrombosis without increasing the risk of bleeding.<sup>12</sup> These key studies led authors to develop a vaccine against TPR that could potentially be used in humans. The mice vaccinated with C-EL2 peptides developed C-EL2Ab, which was shown to specifically inhibit TPR (Figure) as platelets from vaccinated mice aggregated in response to P<sub>2</sub>Y<sub>12</sub> and thrombin receptor agonists; however, platelet aggregation was significantly abolished with TPR agonist U46619. Using an antibody as a therapeutic against thrombosis is not new as the monoclonal antibody abciximab against αIIbβ<sub>3</sub> integrin has been shown to inhibit platelet aggregation but also increases the risk of bleeding and

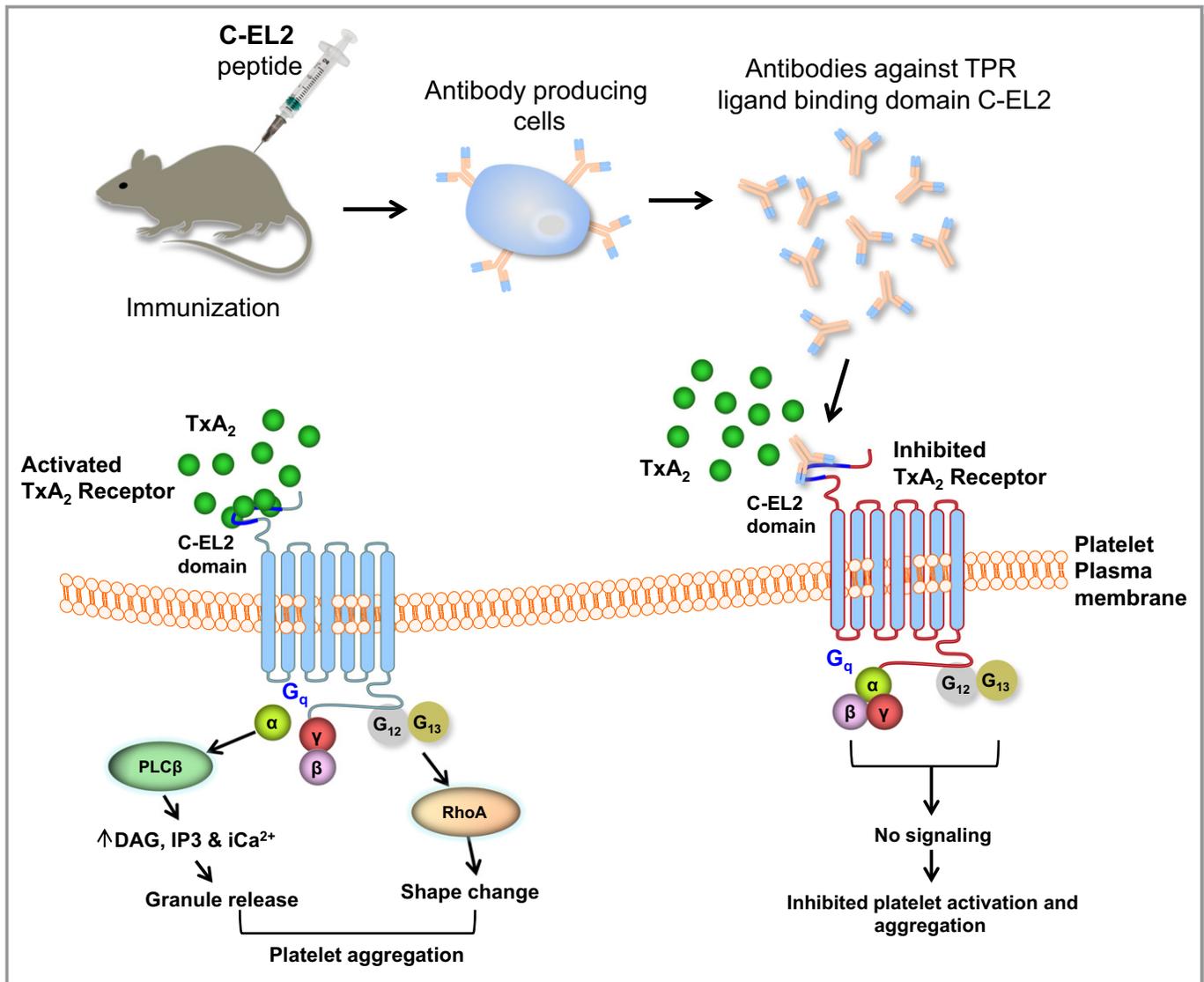
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**Figure.** Schematic representation shows inhibition of thromboxane A<sub>2</sub> receptor and mediated signaling through vaccination. The mice were immunized with C-EL2 peptides, which triggered antibody production against the C-EL2 domain of the TPR. These antibodies bind with C-EL2 domain and block the ligand binding site at the TPR. Left, Activated TPR, which binds with TxA<sub>2</sub> and leads to downstream signaling pathways and platelet aggregation. Right, Inhibited TPR through C-EL2-specific antibody. Blue line in the extracellular domain of the TPR shows the ligand binding site C-EL2. C-EL2 indicates C-terminus of the second extracellular loop; DAG, diacylglycerol; iCa<sup>2+</sup>, intracellular Ca<sup>2+</sup>; IP<sub>3</sub>, inositol triphosphate; TPR, thromboxane A<sub>2</sub> receptor; TxA<sub>2</sub>, thromboxane A<sub>2</sub>; PLCβ, phospholipase C β; RhoA, Ras homolog gene family, member A.

thrombocytopenia compared with traditional therapies.<sup>13</sup> The vaccination approach against TPR is unique and novel and, most importantly, was found to have a minimal impact on hemostasis.

The reported evidence suggests that vaccination often modulates platelets and other blood cell counts.<sup>14,15</sup> One would thus expect a similar effect of TPR vaccine on blood cells. To address this issue, Alshbool et al have assessed the blood counts at 1, 2, and 3 months after a booster dose of C-EL2 vaccine and observed no differences in blood counts between vaccinated and nonvaccinated mice,

although the antibody titers in the vaccinated mice remained elevated.

The vaccine was found to exert no prominent effects on expression of major tested platelet surface receptors including GP Ib, VI, and integrin αIIbβ<sub>3</sub>; however, platelets from vaccinated mice displayed aberrant granule release and αIIbβ<sub>3</sub> integrin activation specifically in response to TPR agonist. These data suggest that the vaccine affects TPR-mediated outside-in and inside-out platelet signaling, both of which are crucial for platelet activation and plug formation through the recruitment of additional platelets. Furthermore,

TPR activation was reported to promote platelet–leukocyte complex formation, which plays a significant role in thrombosis.<sup>16</sup> It is thus expected that TPR vaccine would modulate platelet–leukocyte complex formation. Indeed, the authors found that vaccination inhibits platelet–leukocyte complex formation in response TPR agonist but not with thrombin receptor agonist, which further attests to the specificity of the vaccine against TPR.

Two isoforms of TPR,  $\alpha$  and  $\beta$ , share identical extracellular domains. The  $\alpha$  isoform mainly expresses on platelets, whereas the  $\beta$  isoform largely expresses on endothelial cells.<sup>17</sup> Although the specific function of the  $\beta$  isoform on endothelial cells remains unclear, it is important to characterize the potential effects of this vaccine on modulation of endothelial cell functions. As the authors postulated, the C-EL2 vaccine could potentially be superior over classical antagonists, as antibody remains in the blood circulation and has poor ability to penetrate the endothelial cell layer.<sup>18</sup> Similarly, the vaccine would potentially have a minimal impact on TPR of the smooth muscle, which is known to influence bleeding on injury. In complete agreement with these notions, C-EL2–vaccinated mice displayed normal bleeding time.

It is well documented that sustained elevated levels of intracellular Ca<sup>2+</sup> help platelets to switch from a proadhesive to a procoagulant state.<sup>1</sup> This conversion is mediated by phosphatidylserine (PS) exposure from the inner to the outer plasma membrane, which facilitates thrombin/fibrin generation. TxA<sub>2</sub>- and TPR-mediated signaling induces inositol triphosphate–mediated Ca<sup>2+</sup> store release,<sup>19</sup> and it is expected that the C-EL2 vaccine would potentially alter the Ca<sup>2+</sup> store release and, thus, store-operated Ca<sup>2+</sup> entry. Nevertheless, it is not clear whether vaccination alters the intracellular levels of Ca<sup>2+</sup> and PS exposure, and consequently affects the thrombin/fibrin generation, which is key for hemostasis. Addressing these issues in future studies would strengthen the findings, and the role of a vaccine in modulating the platelet procoagulant activity and bleeding risk would be more clear.

Taken together, the work from Alshbool et al<sup>4</sup> is an important step toward the development of a potential vaccine against TPR. The vaccine would potentially provide complete inhibition of platelet TPR. Most importantly, the TPR-based vaccine was shown to have minimal impact on platelet counts and hemostasis in mice which makes it unique over other traditional antiplatelet agents being used in the clinic. Although the C-EL2 vaccine was well characterized in the mouse model for safety and efficacy and holds promise, the vaccine's toxicity needs to be assessed thoroughly in larger animal models.

## Disclosures

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