in vivo secrete VWF by the constitutive-like route, this could account for a significant portion of circulating plasma VWF. Disruption of VWF secretion has been implicated in Type 2A von Willebrand disease, while targeted disruption of one (or both) pathways could be useful in some prothrombotic states, such as thrombotic thrombocytopenic purpura. Whether continuous VWF secretion is called constitutive, constitutive-like, or basal, the insightful work of Giblin and colleagues raises questions that warrant further investigation.

Conflict-of-interest disclosure: The authors declare no competing financial interests.

REFERENCES

Comment on Olsson et al, page 1078; Stasi et al, page 1147; and Yu et al, page 1325

ITP three R’s: regulation, routing, rituximab

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Tregs create a controlling milieu that promotes immune suppression and autoimmune tolerance. Their versatility and adaptability makes them the “multitalented masters of immune regulation.” In this issue of Blood, 3 studies have shown this concept to be true in patients with chronic ITP. Collectively, the studies show that functional deficiencies of Tregs are responsible for the immune etiology of ITP and that targeting them may alleviate the disease.

Our immune system has elegantly evolved to arm us with many mechanisms that destroy invading microorganisms or stop the spread of tumors. Our system also has extensive built-in mechanisms for preventing attack on healthy self tissues. This “self-tolerance” mechanism involves the elimination of self-reactive T and B lymphocytes during selection in the thymus and bone marrow, respectively. However, since these central mechanisms are not absolute, we have evolved peripheral mechanisms to deal with immune cells that “escape” central tolerance. Over the past 50 years, immunologists have postulated the existence of suppressor T cells that police the peripheral immune system to stop unwanted self immune responses. But this postulated entity was cast into doubt because of hard-to-reproduce model systems, each with complexities and idiosyncrasies. With an explosion of biochemical and molecular advances, however, the field of immune suppression has been resurrected Phoenixlike, and the notion of T regulatory cells (Tregs), marked by specific cell surface molecules (eg, CD4+ CD25+ Foxp3+), arose. Tregs appear to be a relatively rare CD4+ T-cell subset that comprise many subpopulations, including IL-10–producing “Tr1” cells, TGF-β–producing Th1 cells, CD8+ T suppressor cells, natural killer T cells, CD4-CD8–T cells and γδ T cells. Some of these cells originate in the thymus during ontogeny and are referred to as “natural” Tregs. Tregs can also be induced from naïve T cells in the periphery. These Tregs appear to be the natural immune “magic bullets” that keep all of our normal and abnormal immune responses in check. They are critical to our survival, and absence of them can lead to either autoimmunity or inflammatory disorders, with fatal consequences in both mice and humans. Although defects of these cell types have previously been described in immune thrombocytopenic purpura (ITP), 2,3 3 papers in this issue collectively suggest that a deficient Treg compartment allows enhanced T-cell and B-cell autoreactivity in ITP, and that therapies like rituximab actually correct the deficiency and reverse autoimmune platelet reactivity, thus leading to increased platelet counts.

The first study in this series was based on the authors’ previous observations that rituximab (Rituxan) is an efficacious therapy for patients with ITP and that this is due to normalizing abnormal autoreactive T-cell responses in ITP. Stasi and colleagues have extended these intriguing results to show that rituximab primarily reverses the Treg deficiency in patients with ITP. The authors studied 26 adult patients with chronic ITP (a different cohort from their last paper) who were treated with rituximab; they examined Tregs by flow cytometry and assessed their regulatory function by cell proliferation assays. Compared with control individuals, pre-treatment patients with ITP had a significantly reduced number and defective suppressive capacity of Tregs. In addition, the Tregs in the patients with ITP showed a polyclonal spectrum. In contrast, upon treatment with rituximab, patients, particularly responders, showed restored numbers of Tregs as well as restored regulatory functions. The authors suggested that patients with active ITP have a defective Treg compartment, which can be significantly modulated by a B-cell targeted therapy.

In the second paper, Yu and colleagues studied 17 patients with chronic ITP and tested the frequency and functional capabilities of CD4+ CD25+ Foxp3+ Tregs in peripheral blood. Although they found a similar frequency of Tregs in controls and patients, the ability to functionally suppress in vitro T proliferation was significantly reduced in ITP patients. These data further support the notion that functional defects in Tregs probably contribute to a breakdown in self-tolerance in chronic ITP.

In the third paper, Olsson and colleagues studied the bone marrow and peripheral blood of 26 patients with chronic ITP and found, particularly in the bone marrow, increased numbers of infiltrating activated CD3+ T cells with elevated surface expression of VLA-4 and CX3CR1. Compared with controls, the increased T-cell number in the bone marrow was also associated with significantly lower numbers of bone marrow Tregs. They suggest that chronic ITP is a disease of increased activated T-cell due to a Treg deficit within the bone marrow and that this may contribute to suppressed megakaryocyte production in ITP. The 3 studies collectively shed light on how Tregs may initiate and/or mediate the autoimmunity of ITP. It appears that a central deficiency of Tregs breaks tolerance and allows unchecked activation of autoreactive Th1...
cells and B cells. These autoreactive cells, in turn, react against platelet autoantigenic targets, leading to the production of antiplatelet antibodies and cytotoxic T cells. Like autoantibodies, the activated T cells can route to the bone marrow and additionally promote suppression or killing of megakaryocytes. Therapies that either directly or indirectly affect the Treg compartment, as in the case of rituximab, can rescue tolerance and inhibit the abnormal platelet autoreactivity, thereby raising platelet counts. These papers are important not only because they confirm that defective Tregs are at the heart of the autoimmune dysregulation in ITP but because they suggest that development of therapies targeted at Tregs may be the best way to significantly and permanently control the disease.

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Ah receptor: xenobiotic response meets inflammation

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AhR mediates a broad range of biological responses to environmental chemicals, including suppression of immune functions. In this issue of Blood, Lawrence and colleagues demonstrate that AhR is responsible for the anti-inflammatory activity of new antiallergic drug candidates, likely by blocking dendritic-cell function to generate proinflammatory T-helper cells.

Xenobiotic response is a set of genetic programs evolutionarily specialized to defend against xenobiotics—chemicals that humans encounter in the environment. Xenobiotic response is controlled by a group of ligand-activated transcription factors known as xenobiotic-activated receptors (XARs), such as the aryl hydrocarbon receptor (AhR). To mitigate chemical insults, XARs integrate a broad spectrum of biological functions ranging from chemical sensing, drug metabolism, and antioxidative function to immune response, inflammation, and tissue repair, all by regulating the expression of key mediators of a response. The interplay between XARs and immune/inflammatory functions is a particular one: very often, it provides critical protection against noxious chemicals and some microbes, and on occasion, it contributes to the pathogenesis of tissue damage and disease. In both scenarios, it serves as a potential therapeutic target.

AhR was initially identified as the receptor mediating the induction of CYP1A1, the P450 mono-oxygenase critical for metabolic activation of benzo(a)pyrene and 3-methyl-cholanthrene, carcinogens present in tobacco smoke and charcoal-broiled meat.1 AhR gained even wider notoriety after it was found to be required for most, if not all, adaptive and toxicological responses to a large group of widespread, man-made, environmental contaminants—the chlorinated aromatic hydrocarbons, 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD, dioxin) is the most potent agonist of AhR, causing a wide range of biological responses including thymic involution and suppression of immune and inflammatory responses, providing a direct link between AhR and immune/inflammatory functions. Although AhR ligands have been developed as antiancancer drugs,2 the therapeutic potential of AhR in immune and inflammatory diseases has not been explored until now.

In this issue of Blood, Lawrence and colleagues describe a series of experiments demonstrating that AhR is required for both the in vivo and in vitro anti-inflammatory effects of a drug candidate, VAF347, which inhibits allergic lung inflammation. The initial clue of a connection between AhR and the immune-modulating drug came unexpectedly. In a search for molecular targets via RNA chip analysis, VAF347 was found to induce AhR target genes AhRR, CYP1B1, and TipARP, in addition to down-regulating IL-6 as expected; it also altered expression of several other genes in immature and anti-CD40–activated, monocyte-derived dendritic cells (DCs), which are critical in the development of T-helper (Th) cells and immune responses. Through a series of molecular and biochemical studies, the authors prove that VAF347 is indeed an agonist of AhR: it binds to AhR with a high affinity, activates AhR in vitro, and induces CYP1A1 in human peripheral monocytes, similarly to the prototypical agonist of AhR, TCDD. Conversely, TCDD was shown in the studies to inhibit IL-6 production by mature monocyte-derived DCs and to block DC-mediated autologous T-cell proliferation, in parallel with VAF347. On the other hand, VAG005, an inactive derivative of VF347, was only weakly bound to AhR and had no effect on the functions measured. These findings reveal a clear correlation between AhR binding and the immune-modulating activity of the agents in vitro; a causal relationship between the 2 was subsequently proven using genetic interventions. In cultured cells, a truncated AhR (AhR515, a dominant-negative form) was introduced into the human monocyctic cell line MonoMac1 (MM1); overexpression of AhR515 was found to block VAF347 or
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