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Tregitopes switch on Tregs

Rachel R. Caspi

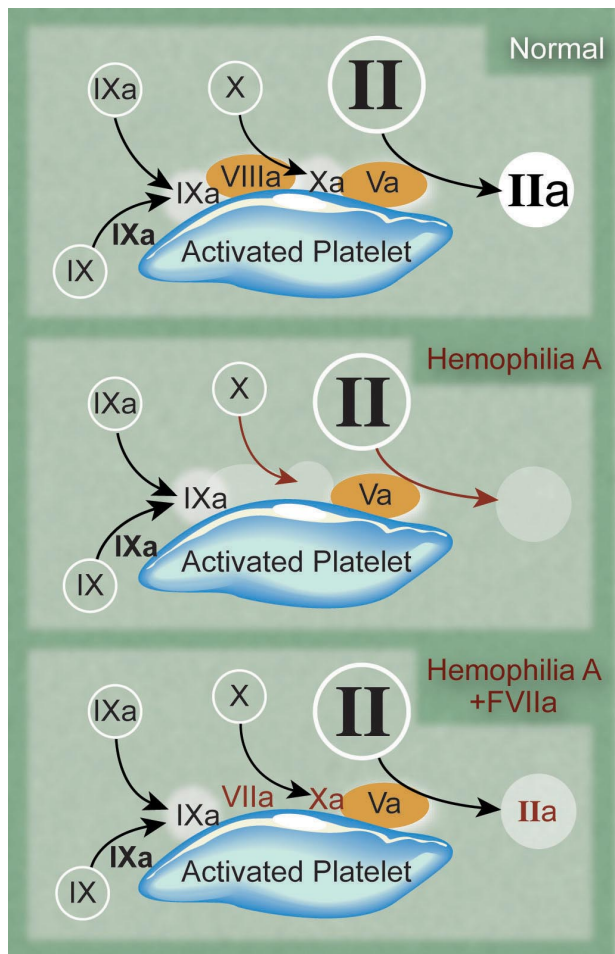
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This second figure is of thrombin generation on platelet surfaces. In normal hemostasis, FIXa combines with FVIIIa on the activated platelet surface to activate FX. The FXa, in turn, combines with FVa to produce thrombin. In severe hemophilia, there is no platelet surface mechanism to activate FX. When high-dose FVIIa is used as a bypassing agent, it binds to the activated platelet surface and activates small amounts of FX. The resulting FXa is able to partially restore platelet surface thrombin generation. The binding and activity of FVIIa is mediated by negatively charged phospholipid, the GPIb/IX/V complex, and possibly other binding proteins that are unrecognized as of yet. Professional illustration by Debra Tyler Dartz.

and targeted forms of VIIa as future hemostatic agents.

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Immunoglobulin (Ig) has long been known to have tolerogenic properties. Thus, antigens (Ags) conjugated to Ig elicit tolerance rather than immunity,^{1,2} and intravenous administration of pooled Ig from multiple donors, known as intravenous immunoglobulin (IVIg), is used in clinical practice to treat autoimmune and inflammatory diseases.³ The reason for these tolerogenic effects of Ig is not understood, but recently IVIg has been shown to enhance human regulatory T cells (Tregs).^{4,5} This, together with the observation that Fc fusion proteins of soluble receptors and other bioactive molecules are either poorly or nonimmunogenic, and antibody (Ab) variable regions (to which central tolerance should not exist) do not elicit robust autoimmune responses, led De Groot et al to postulate that the Ig molecule must contain regions or epitopes that are stimulatory to Tregs (ie, Tregitopes).

Using computational epitope mapping, the authors looked for consensus 9 amino acid regions in the human Ig molecule that would bind to multiple HLA class II molecules (on the premise that most Tregs are CD4-restricted). They identified 2 such clusters of major histocompatibility complex (MHC) binding motifs in the Fc molecule that could be presented to T cells. Predicted human Tregitope (hTregitope) sequences 167 and 289 were synthesized and were indeed shown to bind to multiple MHC class II molecules. Using a variety of Ags and culture conditions, the authors presented evidence that these Tregitope peptides activate as well as expand Tregs. The authors conclude that both natural Tregs (nTregs) and Ag-specific adaptive Tregs are affected. However, due to limitations of the experimental setup and the complexities of the human system, the distinction between effects on natural versus adaptive Tregs (as in humans, CD4⁺CD25^{high} cells are a mixture of both) and between the expansion of preexisting FoxP3⁺ cells versus their de novo conversion from conventional T cells is not always clear.

In the next step, the functional effects of Tregitopes on Ag-induced cytokine production and surface activation markers are documented using depletion experiments and Ag-MHC tetramers. The authors use a pool of immunogenic peptides derived from the complement component C3d (an autologous

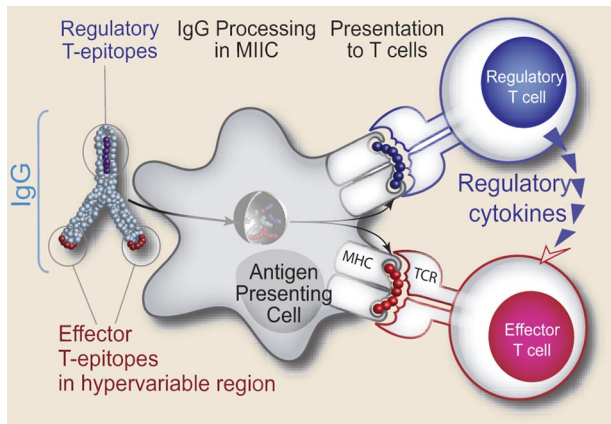
IMMUNOBIOLOGY

Comment on De Groot et al, page 3303

Tregitopes switch on Tregs

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In this issue of *Blood*, De Groot and colleagues report the identification and functional characterization of Tregitopes, which are Treg-activating regions in the Fc portion of the IgG molecule. This important finding has the potential to bring understanding about a number of phenomena related to Ig, including tolerance to Ab variable regions, the tolerogenic properties of immunoglobulin-Ag conjugates, the weak immunogenicity of Fc fusion proteins, and the therapeutic and regulatory effects of clinical preparations of IVIg on autoimmune and inflammatory diseases.



Hypothesized tolerizing mechanism of IgG. Conserved T-cell epitopes in IgG that engage nTregs have been discovered. The authors hypothesize that antibody-derived Treg epitopes (dark blue epitope) activate Tregs, leading to suppression of effector T cells that recognize effector epitopes (red epitope), like those of IgG hypervariable regions to which central tolerance does not exist. Whether this suppression is mediated by regulatory cytokines alone, or whether contact-dependent signaling also plays a role, has yet to be determined. See the complete figure in the article beginning on page 3303.

T-cell target) and birch pollen allergen tetramers to demonstrate that, in the presence of Tregitopes, the proinflammatory and allergic responses are attenuated, whereas the anti-inflammatory cytokines are enhanced. Similarly, surface markers associated with regulatory function are enhanced, whereas markers associated with effector function are attenuated. Finally, the authors support their studies in vivo by using HLA-Tg mice immunized with house dust mite allergen and showing that coadministration of the murine equivalents of hTregitopes attenuates induction of responses to house dust mite allergen.

The authors hypothesize that Ab-derived Tregitope sequences presented on MHC class II⁺ Ag-presenting cells activate Tregs, leading to

down-regulation of effector cell activation and function via regulatory cytokines, as shown in the figure. The model does not take into account Ag dependence on activation/expansion of Ag-specific adaptive Tregs and does not address (but does not negate) the reported role of cell to cell contact in suppression by nTregs. Although it leaves open many questions, this is an important paper that helps to shed light on the well documented but poorly understood phenomena associated with the tolerogenic effects of immunoglobulins.

Conflict-of-interest disclosure: The author declares no competing financial interests. ■

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NEOPLASIA

Comment on Zöllinger et al, page 3403

Need akt? Some myelomas do

P. Leif Bergsagel MAYO CLINIC

The signaling pathway vivisection of MM by Zöllinger and colleagues has elegantly isolated the Akt pathway using specific genetic and pharmacologic inhibitors and shown that some but not all MMs are critically dependent on akt signaling.

The signaling alchemists are searching for the philosopher's stone that divines a base signal for normal cells as a critical signal for tumor cells. Unfortunately, there appears to be a hopelessly intricate web of diverse signaling pathways (eg, Akt, MAPK, STAT3),

many of which have previously been implicated as critical for the survival of multiple myeloma (MM). Instead of presenting a confusogram that unsuccessfully attempts to diagram and reconcile this complexity, the authors have adopted a reductionist approach

and dichotomized MM into those that depend on Akt signaling and those that do not. The important clinical implication to their work is that some fraction of patients with MM will be expected to respond to treatment with an Akt kinase inhibitor, such as the selective, non-ATP-competitive inhibitor (Akti-1/2) used in their studies. Importantly, in order to identify patients with sensitive tumors, Zöllinger et al use a clinically useful marker: levels of phospho-Akt in MM cells detected by immunohistochemistry or flow cytometry.

Although the authors exclude genetic inactivation of PTEN, further studies will be required to define the mechanism underlying the Akt activation seen in MM. The fact that it is seen in some but not all MM suggests that it does not represent a feature of normal plasma cell biology and more likely represents a tumor-specific transforming event. Intriguingly, the simultaneous analysis of multiple signaling pathways (Akt, MAPK, STAT3) in primary MM cells has shown that several tumors independent of Akt were likewise independent of MAPK and STAT3. The identities of the signals, if any, on which these latter tumors depend remain to be identified.

The limitations of this study are common to all preclinical functional studies in MM. The field is severely limited by the lack of faithful preclinical models, and in particular, by the inability to culture primary MM cells in vitro. Assays performed on primary cells, either alone or in contact with stromal cells, are restricted to the measurement of a change in the intrinsic rate of apoptosis that begins from the moment the tumors cells are extracted from the patient. It is not possible to routinely stimulate proliferation or expansion of primary cells in vitro. Although the use of stromal cells is helpful, it is not possible to duplicate in vitro the complex microenvironment of a human bone marrow. Myeloma cell lines are useful tools for discovery, but they differ significantly from primary tumor cells. Not only are they much more proliferative, but they also contain a variety of additional genetic changes, some of which are quite uncommon in primary patient tumors (eg, mutational inactivation of PTEN). Finally, the authors have not performed xenograft studies, in which the same cell lines that are responsive in vitro are grown and treated in immunodeficient mice. These studies would not have helped to define the role of the Akt pathway in MM, the main point of this paper, but would have spoken to the