

The TWEAK/Fn14 axis in anaphylactic shock



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Anaphylaxis, described more than 100 years ago, remains the immune response that requires the most rapid, and definitive, treatment to prevent death.¹ Moreover, the sudden onset of severe anaphylaxis may be the first clue that a person is susceptible to this catastrophic and potentially life-threatening reaction.² And, remarkably, the most effective treatment for severe anaphylaxis is intramuscular epinephrine, and this has been true for more than 40 years.¹⁻³

Given the serious threat posed by anaphylaxis, considerable effort has gone into attempting to explain the phenomenon mechanistically, ideally in a way that also suggests how to improve therapy. In this issue of the *Journal*, Mendez-Barbero et al⁴ describe, primarily on the basis of studies in mice, the involvement in anaphylaxis of the TNF-like weak inducer of apoptosis (TWEAK) and its sole receptor, the fibroblast growth factor-inducible molecule 14 (Fn14). TWEAK, which can be produced by monocytes and many other cells,⁵ has been shown to contribute to skin inflammation in mouse models of atopic dermatitis and psoriasis.⁶ In the present study, the authors compared expression of mouse models of passive and active systemic anaphylaxis in wild-type C57BL/6 mice and in C57BL/6 mice deficient in *TWEAK* or *Fn14*. The authors also interrogated primary bone marrow-derived cultured mast cells (BMCMCs) and endothelial cells derived from wild-type and *TWEAK*^{-/-} or *Fn14*^{-/-} mice.

The authors showed that passive or active systemic anaphylaxis substantially increased lung levels of Fn14 and TWEAK, as well as serum-soluble concentrations of TWEAK (sTWEAK).⁴ More importantly, mice deficient in either TWEAK or Fn14 exhibited significant reductions (but not an absence) of passive anaphylaxis-related signs, namely, reductions in hypothermia and less severe impairment of physical activity. Significantly, the authors demonstrated that genetic loss of TWEAK or Fn14

did not influence the number of mast cells in several tested tissues. Furthermore, although they showed that BMCMCs could secrete sTWEAK and increase Fn14 on 2 hours of FcεRI aggregation *in vitro*, there was no significant difference in BMCMC degranulation, or in histamine, IL-6, or TNF release, in wild-type, *TWEAK*^{-/-}, or *Fn14*^{-/-} BMCMCs after stimulation *in vitro* with IgE and specific antigen.

However, the administration of histamine or PAF (*in vitro* or *in vivo*) increased the expression of Fn14 in lungs and in mouse aortic endothelial cells. When stimulated *in vitro* either with histamine or with PAF, or by activated wild-type BMCMCs, the aortic or lung endothelial cells of wild-type mice, but not those of Fn14-deficient mice, exhibited enhanced permeability. This ability of wild-type BMCMCs to stimulate enhanced endothelial cell permeability *in vitro* was not observed when *TWEAK*^{-/-} BMCMCs were tested. Finally, they showed that genetic deletion of TWEAK or Fn14, or administering a TWEAK-blocking antibody, reduced by about 50% the ability of histamine or PAF to enhance subcutaneous vascular permeability.

This new evidence for a role for mast cell-derived TWEAK and endothelial cell-associated Fn14 in IgE-dependent anaphylaxis in C57BL/6 mice appears sound; however, more work will be required to extend the work to human anaphylaxis. The authors analyzed plasma specimens from 24 control subjects and 55 patients with anaphylaxis, but the ages and sexes of the control subjects were not reported. Nor was it stated exactly when in relationship to the last anaphylactic episode the specimens from subjects with anaphylaxis were obtained. This might account for the broad overlap of sTWEAK levels in the control and anaphylaxis subjects, despite the *P* = .01 difference in the groups (Fig 7, A). It might also have influenced the lack of significant correlation of the severity grades of the anaphylactic episodes and sTWEAK levels (*P* = .128).

Indeed, comparison with the mouse data shows a much better separation of sTWEAK values between control mice and mice with active or passive systemic anaphylaxis (Fig 1, F) than for human controls and anaphylaxis subjects (Fig 7, A). Also, although Fig 7, C, shows virtually no overlap in Fn14 values between control and anaphylaxis subjects when their serum was used to stimulate human dermal microvascular endothelial cells for 2 hours *in vitro*, the comparison was between serum from only 4 control and 7 anaphylaxis subjects, and the demographic information regarding this small set of subjects was not provided. Thus, the relationship of TWEAK and Fn14 to anaphylaxis in humans requires considerably more work. This point is acknowledged by the authors, who wrote: "Though a comprehensive study designed to elucidate the usefulness of sTWEAK in human sera anaphylaxis samples would be highly beneficial, investigations of this nature face several complications due to the numerous triggers, mechanisms, severities, and the difficulty of obtaining homogenous samples."⁵

This is a wise general expression of the difficulties to be anticipated in extending findings obtained during carefully controlled experiments in a single strain of inbred mice to the

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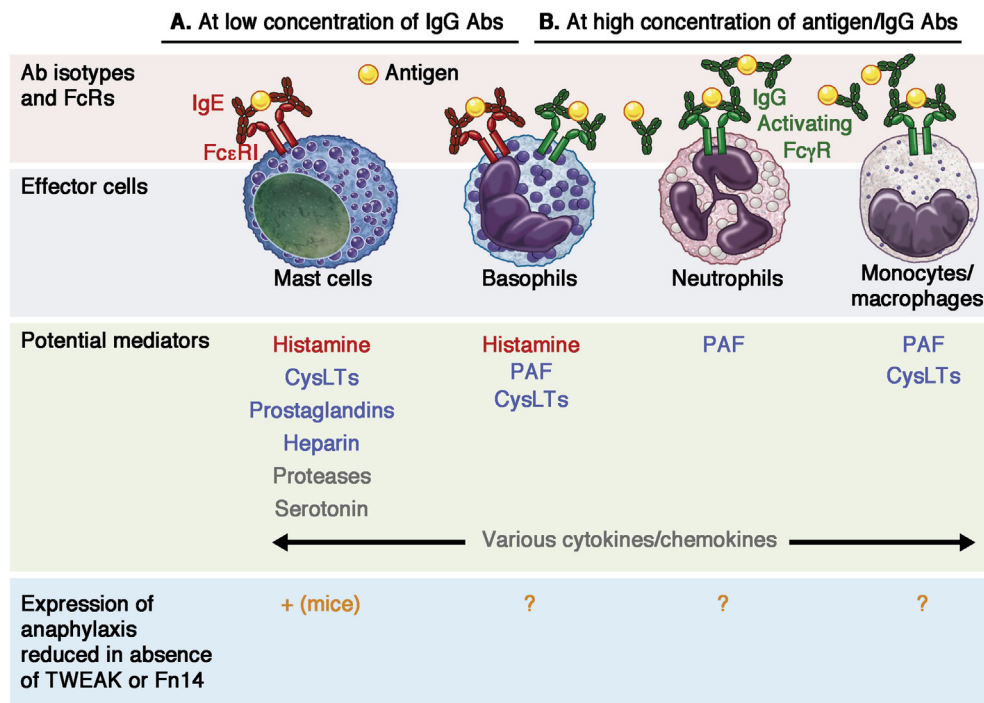


FIG 1. Multiple potential pathways in antibody (Ab)-mediated anaphylaxis, and their susceptibility to participation of TWEAK and Fn14. **A**, Antigen-specific IgE antibodies and FcεRI-bearing effector cells (eg, mast cells and basophils) play a dominant role in anaphylaxis induced (sometimes by very small amounts of bivalent or multivalent antigen) when concentrations of IgE antibodies are low. **B**, Mouse models of anaphylaxis suggest that IgG antibodies and FcγR-bearing effector cells (eg, basophils, macrophages, neutrophils, and mast cells) can be important effectors of anaphylaxis induced by large amounts of antigen that forms immune complexes in the presence of high concentrations of IgG antibodies. Some examples of anaphylaxis likely involve both pathways (Fig 1, A and B). Note that coengagement of immunoreceptor tyrosine-based activation motif-containing activating FcγRs or FcεRI with the immunoreceptor tyrosine-based inhibitory motif-bearing FcγRIIB (on mast cells [in mice, but perhaps not, or at lower levels, in human subjects] or basophils [in human subjects and mice]) can act to diminish effector cell activation. Red indicates strong evidence for the importance of these mediators in human anaphylaxis induced by antigen. Blue indicates that these elements can participate in models of anaphylaxis in mice, but their importance in human anaphylaxis is not yet clear. Gray indicates elements with the potential to influence anaphylaxis, but their importance in human or mouse anaphylaxis is not yet clear (eg, human mast cells are thought to make little or no serotonin). Mendez-Barbero et al⁴ have demonstrated an important role for TWEAK/Fn14 in mice in the histamine- and PAF-mediated changes associated with IgE-associated anaphylactic shock. CysLTs, Cysteinyl leukotrienes.

human condition(s) being modeled. Indeed, one should consider the immunologic complexity of conditions that are lumped under the rubric “anaphylaxis” in humans. For example, Jönsson et al⁷ recently reported strong evidence that human drug-induced anaphylaxis can reflect the participation of an IgG-induced neutrophil activation pathway. The group conducted a multicentric study of 86 patients with suspected anaphylaxis to neuromuscular-blocking agents (NMBAs) administered during general anesthesia and also analyzed 86 matched controls. They found that the concentrations of IgG anti-NMBA antibodies, as well as markers of FcγR activation, PAF release, and neutrophil activation, correlated with the severity of anaphylaxis. Moreover, such neutrophil activation was observed in some patients who lacked detectable evidence of classical IgE-dependent anaphylaxis.

These, and other, findings support the involvement of an IgG-neutrophil pathway in NMBA-induced anaphylaxis in humans. They also suggest that this mechanism may enhance anaphylaxis in combination with an IgE-dependent mechanism or may

actually underlie the anaphylaxis observed in the absence of detectable antigen-specific IgE.⁷ Of course, both IgE and IgG also can contribute to the expression of anaphylaxis in C57BL/6 mice.^{8,9} However, the extent to which mice deficient in either TWEAK or Fn14 might be able to express IgG-dependent anaphylaxis remains to be tested (Fig 1).

Even if one considers solely IgE-dependent anaphylaxis in humans, the complexity of the immune mechanisms underlying this disorder continues to be elucidated. For example, Gowthaman et al¹⁰ recently identified a T follicular helper cell subset (which they termed “T_{FH}13” cells) that drives production of anaphylactic IgE. Such “T_{FH}13” cells (with the cytokine profile IL-13^{hi}, IL-4^{hi}, IL-5^{hi}, IL-21^{lo}) and that coexpress the transcription factors BCL6 and GATA3 are required for production of high-affinity IgE and subsequent allergen-induced anaphylaxis. The authors propose that “Blocking T_{FH}13 cells may represent an alternative therapeutic target to ameliorate anaphylaxis.”¹⁰ Again, although such approaches may be applicable to cases of anaphylaxis caused by high-affinity IgE,

it is not clear whether they would be effective against cases that are not IgE-associated.

The mouse has several advantages, including the availability of inbred animals with defined genetic alterations, which can reveal a comprehensive picture of how immunity works in that species. This certainly has been true regarding the mouse's role in helping to elucidate the mechanisms of anaphylactic reactivity. But showing the relevance of findings obtained in inbred mice for outbred humans with anaphylaxis is complicated. And people can die of anaphylaxis within minutes of its onset.¹⁻³ Accordingly, whether treatment of human anaphylaxis with epinephrine will soon be superseded by alternative forms of therapy remains to be seen.

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