inside blood

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PLENARY PAPER

Comment on Stanzani et al, page 2258

Mouldy oldy: how fungus lives among us

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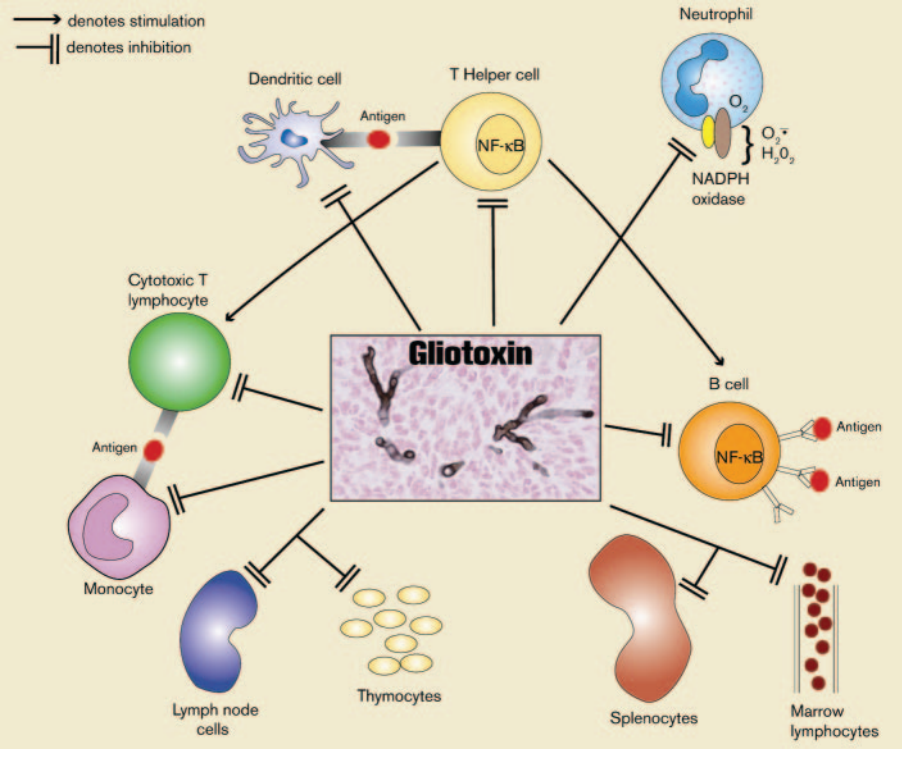
The work by Stanzani and colleagues illustrates the prescience of the statement by Jonathan Swift, "Hobbes clearly proves that every creature lives in a state of war by nature,"[1] when applied to weaponry used by opportunistic fungi to survive in mammalian hosts. *Aspergillus fumigatus* produces a variety of proteases, reactive oxidant scavengers, phospholipases, and hemolysins that mediate penetrating the lung epithelial barrier, nutrient acquisition, and evasion of host defense.

Gliotoxin, an abundant toxin produced by *Aspergillus fumigatus* (AF) and other fungi, belongs to the epipolythiodioxopiperazine class of metabolites. Gliotoxin has broad immunosuppressive properties that facilitate evasion of host defense (Figure 1). It inhibits phagocyte nicotinamide adenine dinucleotide phosphate (NADPH) oxidase assembly and activation, a key component in host defense against filamentous fungi.[2] Gliotoxin also inhibits ingestion of AF by macrophages,[3] inhibits cytotoxic T-lymphocyte cytotoxicity,[3] induces apoptosis of thymocytes, splenocytes, and mesenteric lymph node cells,[4] selectively depletes bone marrow of mature lymphocytes in mice, and inhibits activation of nuclear factor kappa B (NF-κB; a transcriptional factor that is a key mediator of cytokine and inflammatory responses) in T and B cells.[5]



Gliotoxin, a toxin produced by AF, suppresses several key pathways in innate and antigen-specific immunity. Gliotoxin likely suppresses T-cell responses principally by inducing apoptosis of antigen-presenting cells. The center box depicts experimental invasive pulmonary aspergillosis in a mouse (silver stain; original magnification × 400).

In this issue of *Blood*, Stanzani and colleagues made a significant contribution to further understanding the immunosuppressive properties of gliotoxin. They showed that $CD4^+$ and $CD8^+$ T cells from healthy donors had weak cytokine responses to AF crude filtrates, a paradoxical finding given that we are regularly exposed to this ubiquitous organism. Gliotoxin suppressed functional T-cell responses to cytomegalovirus (CMV) and the superantigen staphylococcal enterotoxin B, principally by inducing apoptosis of monocytes and dendritic cells. The effects of gliotoxin in vitro occurred at concentrations below those observed in the serum of patients with invasive aspergillosis. This study highlights the potential immunomodulatory properties of gliotoxin during invasive aspergillosis and raises the possibility of gliotoxin-mediated immunosuppression predisposing such patients to additional opportunistic infections, such as CMV.

Casadevall et al[6] employed the term "dual use" virulence factors to describe factors that confer a survival advantage to pathogens both in the environment and in animals. For example, the virulence of *Cryptococcus neoformans* in animals may have originated from factors that enabled persistence in amoeboid and nematode predators. AF thrives in the environment, but its ecologic niche in humans is highly restrictive, generally only afflicting the severely immunocompromised. It would be useful to understand what other function (if any) gliotoxin serves

in the natural environment and whether gliotoxin production correlates with virulence. Potentially, clinical isolates of AF may be more robust gliotoxin producers than environmental strains. Generation of knockout strains deficient in gliotoxin production would be more definitive in evaluating gliotoxin as a virulence factor in experimental aspergillosis and its role in modulating mammalian host defense.

Knowledge gained about gliotoxin may be exploited in the clinic. Circulating gliotoxin levels may be of value in diagnosing *Aspergillus* infection. Gliotoxin is also a promising target for drug development, and generation of neutralizing antibodies against gliotoxin may be of value in attenuating fungal virulence. In the Hobbesian vision, the more tools we have in our armamentarium, the better. ■

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HEMATOPOIESIS

Comment on Tanaka et al, page 2324

Mutant Kit: thwarting the message down below

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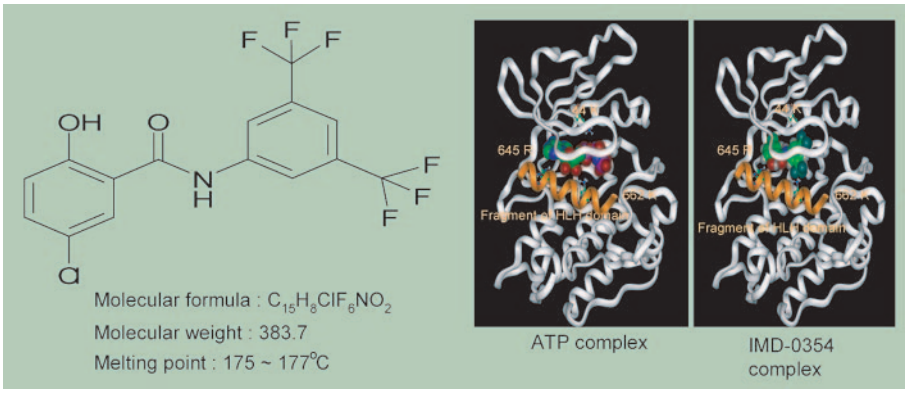
Signal transduction pathways involving Kit^{WT} have been extensively studied, whereas there is limited information regarding mutant Kit signaling. In this issue of *Blood*, Tanaka and colleagues describe the constitutive activation of NF-κB in this regard.

Human Kit is a type III receptor tyrosine kinase, the protein product of the proto-oncogene c-*kit*, which is localized to chromosome 4q12. Stem cell factor (SCF) and wild-type Kit (Kit^{WT}) are considered the key ligand-receptor pair for the growth and survival of normal mast cells. Kit is normally activated by autophosphorylation brought about by ligand-induced dimerization of Kit monomers. Activation of Kit in neoplastic mast cells bypasses SCF through gain-of-function mutations that are usually clustered around the kinase domain (eg, Kit^{Asp816Val}) but which also occur at the juxtamembrane region (eg, Kit^{Val560Gly}) of the receptor. Post-Kit signal transmission in the imatinib mesylate (STI571)–resistant mutant receptor (Kit^{Asp816Val}) has been shown to require activation of phosphatidylinositol 3 (PI3) kinase and, further downstream, that of the serine/threonine kinases Jnk1 and Jnk2 and signal transducer and activator of transcription 3 (STAT3) but not the mitogen-activated protein (MAP) kinase family members Erk1 and Erk2.[1,2] PI3 kinase and STAT3 are also constitutively activated in the murine homologue of the human catalytic domain mutant receptor (Kit$^{Asp814Tyr/Val}$) where an ubiquitin-mediated degradation of the tyrosine phosphatase SHP-1 as well as activation of the δ isoform of protein kinase C (PKCδ) have also been reported.[3-6]



Inhibitory effect of IMD-0354 on cell proliferation of HMC-1 cells. See the complete figure in the article beginning on page 2324.

In this issue of *Blood*, Tanaka and colleagues provide additional information regarding mutant Kit signaling by demonstrating a cell-cycle progression–relevant (ie, associated with cyclin D3 expression) constitutive activation of nuclear factor kappa B (NF-κB), which was accompanied by phosphorylation of its inhibitor (IκB), in the context of both Kit^{Asp816Val} and Kit^{Val560Gly}. Furthermore, consistent with the aforementioned observations, inhibition of either the PI3 kinase or PKC but not the MAP kinase pathway impaired NF-κB activation as well as cell proliferation by mutant Kit. Taken together, this suggested IκB phosphorylation as the essential link between upstream signaling events and NF-κB activation associated with mutant Kit. Consistent with this possibility, NF-κB activation as well as cell proliferation, in the context of both juxtamembrane and catalytic domain Kit mutants, were inhibited by an IκB kinase inhibitor (IMD-0354; *N*-(3,5-Bis-trifluoromethyl-phenyl)-5-chloro-2-hydroxy-benzamide; see figure). It was interesting to note that the drug inhibited NF-κB activation but not cell proliferation in normal human mast cells, which suggested a limited degree of selectivity.

The above set of observations allows one to envision different molecular levels of drug intervention in systemic mastocytosis (SM). At the receptor level, novel dual kinase inhibitors currently being evaluated in clinical trials have already been shown to inhibit Kit^{Asp816Val} in vitro. Alternatively, proteasomal degradation