Ibrutinib blocks Btk-dependent NF-κB and NFAT responses in human macrophages during Aspergillus fumigatus phagocytosis

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Ibrutinib is a small molecule Bruton tyrosine kinase (Btk) inhibitor approved by the Food and Drug Administration for clinical use in the treatment of chronic lymphocytic leukemia, Waldenström macroglobulinemia, and as a second-line treatment of lymphoma and chronic graft-versus-host disease.1 An association with pulmonary aspergillosis was observed shortly after Ibrutinib was licensed for use.2 A recent phase Ib study of Ibrutinib treatment of primary central nervous system lymphoma reported a 39% incidence of invasive aspergillosis, in patients concurrently treated with corticosteroids, in the absence of neutropenia.3 Studies of Aspergillus fumigatus infection in Btk−/− mice revealed focal pneumonia and large airway mucous plugs, mirroring findings in macrophage-depleted models of pulmonary aspergillosis.3

We recently described a key role for Btk in macrophage immune responses during experimental pulmonary aspergillosis.4 Btk was critical for endosomal signaling responses during murine macrophage phagocytosis of A fumigatus. Btk activation led to calcineurin-NFAT signaling, which was crucial for orchestrating neutrophil recruitment during pulmonary aspergillosis and was dependent on the endosomal DNA receptor TLR9. These observations suggest that defects in macrophage Btk signaling contribute to susceptibility to pulmonary aspergillosis. Here we show that Ibrutinib is a potent inhibitor of both NFAT and nuclear factor κ-light-chain-enhancer of activated B cells (NF-κB) responses in human macrophages during infection with A fumigatus. We show that A fumigatus induces human macrophage Btk phosphorylation, and that Btk depletion impairs NFAT and NF-κB responses in human macrophages. Our findings suggest Btk involvement in a TLR9-dependent endosomally driven pathway in accordance with previous findings in our murine model. In addition, our results show that Ibrutinib is a strong inhibitor of macrophage responses to A fumigatus, which may increase the susceptibility of patients on Ibrutinib to invasive aspergillosis.

Peripheral blood samples were collected from unscreened healthy donors. THP-1–derived macrophages, human monocyte–derived macrophages (hMDMs), and alveolar macrophages were isolated and differentiated as previously described.5 The study was approved by the Biomedical Research Unit (National Research Ethics Service reference 10/H0504/9[DAJ3]), Royal Brompton, and Harefield NHS Trust.
phosphorylation of Btk at Tyr 223 determined by western blotting (Figure 1A). Infection induced phosphorylation of Btk, which was blocked by Ibrutinib. In addition, Ibrutinib inhibited A. fumigatus–dependent nuclear translocation of NFAT and NF-κB (Figure 1B). Zymosan, but not C. albicans, was able to induce BTK phosphorylation (supplemental Figure 1, available on the Blood Web site). The role of Btk in NFAT and NF-κB translocation was confirmed by Btk siRNA knockdown during A. fumigatus infection of hMDMs, by confocal microscopy (Figure 1C–D; supplemental Figure 2). Accordingly, both Ibrutinib and Btk siRNA inhibited hMDM and alveolar macrophage TNF-α responses during A. fumigatus infection (Figure 1E–H). These observations indicate that Ibrutinib blocks inflammatory responses to A. fumigatus in human macrophages through a Btk-dependent pathway.

Our murine studies indicated that Btk-dependent macrophage responses to A. fumigatus are mediated through an endosomal TLR9 signaling pathway.4 Consistent with these observations,
A fumigatus–dependent NFAT translocation in hMDMs was blocked by both Ibrutinib and the TLR9-blocking nucleotide ODN2088 (Figure 2A). Furthermore, inhibition of phagocytosis of A fumigatus by hMDMs using cytochalasin D led to a loss of Btk-dependency for NFAT-dependent signaling responses (Figure 2B). In accordance with this finding, cytochalasin D inhibited Btk phosphorylation in hMDMs during A fumigatus infection (Figure 2C). Using confocal fluorescence microscopy, we confirmed that Btk colocalizes with swollen, but not resting, A fumigatus conidia during phagocytosis (Figure 2D). However, Ibrutinib had no inhibitory effect on phagocytosis (Figure 2E).

Ibrutinib impaired fungal growth control by macrophages (Figure 2F). These observations suggest that A fumigatus–dependent macrophage Btk signaling is endosomally driven and dependent on TLR9. Btk has also been shown to regulate reactive oxygen species production, in ammasome activation, and adhesion in myeloid cells. Additionally, Btk has been shown to regulate re- active oxygen species production, in ammasome activation, and adhesion in myeloid cells. Further studies should focus on defining the wider impact of Ibrutinib on TEC kinase-dependent innate immunity to fungi.15

Figure 2. Endosomally driven Btk responses during human macrophage infection with A fumigatus are required for optimal fungal growth control. (A) TLR9 engagement and BTK phosphorylation are required for NFAT activation in response to A fumigatus in hMDMs. hMDMs were pretreated with Ibrutinib (1 μM), the TLR9-blocking nucleotide ODN2088 (10 μM), or ODN nucleotide control (10 μM) for 1 hour. Macrophages were then infected with A fumigatus swollen conidia (MOI = 1) for 1 hour. Whole cell lysates were separated by SDS-PAGE, followed by western blotting. Membranes were probed with anti-NFATc1 and Histone H3 antibodies. (B-C) BTK mediates an endosomal nucleotide ODN2088 (10 μM) or ODN nucleotide control (10 μM) for 1 hour. Macrophages were then infected with A fumigatus swollen conidia (MOI = 1) for 1 hour. Whole cell lysates were separated by SDS-PAGE, followed by western blotting. Membranes were probed with anti-BTK and anti-Histone H3 antibodies. (B) Monocyte-derived macrophages were pretreated with scramble or BTK-targeting siRNA (100 nM) for 72 hours and then stained with BTK (red) and nuclei (blue) (original magnification x60). (a) Control; (b) Scramble; (c) BTK siRNA. (C) Phagocytosis was quantified by calculating the percent overlap of the nuclear DAPI and transcription factor–linked fluorophore channels. Data were calculated from 7 fields of view taken at random per biological repeat. Mean and standard deviation of 3 biological repeats are represented. (D) BTK is recruited to the membranes of endosomes containing swollen but not resting conidia (MOI = 1) for 1 hour. Macrophages were infected with biotinylated A fumigatus swollen conidia (MOI = 1) for 2 hours. External conidia were then counterstained with Cy3 biotin antibody, and phagocytosis was measured by flow cytometry. (F) Ibrutinib impairs macrophage control of fungal growth in vitro. Monocyte-derived macrophages were pretreated with Ibrutinib (1 μM) for 1 hour. Cells were stimulated with A fumigatus swollen conidia (MOI = 1) for 6 hours. Galactomannan levels were measured in the tissue culture supernatants. UT, untreated.
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Authorship

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Footnotes

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REFERENCES


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TO THE EDITOR:

Treatment of AL amyloidosis with bendamustine: a study of 122 patients

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Chemotherapy for light chain (AL) amyloidosis is based on combinations developed for multiple myeloma.1 A better understanding of susceptibility of the AL underlying clone to specific types of treatments2 and the ability to identify cytogenetic patterns with different clinical outcomes3,4 are beginning to change the approach to this rare and still fatal disease. Despite the high response rates to first-line regimens, treatment of relapsed/refractory patients remains an important unmet need.5 Relapsed patients may have a good outcome if treated before organ progression.6,5 Light chain amyloidosis caused by immunoglobulin M–producing clones (IgM-AL amyloidosis) is a distinct clinical entity and poses additional problems in the design of the therapeutic strategy.8 Rituximab- and bortezomib-based regimens developed for Waldenström macroglobulinemia9 have been evaluated in IgM-AL amyloidosis and are considered first-line options for these patients,9,10 and bendamustine is being evaluated in a phase 2 trial in relapsed AL amyloidosis.11

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