The role of monocytes in resolving inflammation in Behçet's Syndrome

Adebowale Adesanya, William Ogunkolade, Amal Senusi, Sarah Sacoor, Bindi Gokani, Azimoon Bibi, Bindi Gokani, Nardos Wakjira, Helen Lock, Fabian Flores-Borja, Farida Fortune

[1]Centre for Oral Immunobiology and Regenerative Medicine, Faculty of Medicine and Dentistry, Queen Mary University of London, UK.
[2]London Behçet's Centre of Excellence, Royal London Hospital, Barts Health London, UK.

Introduction

Behçet's syndrome (BS) is an immune-mediated vasculitis that is clinically reported to be more severe in younger males. The pathogenesis underpinning BD is yet to be fully understood. The core pathogenic response in BS is primarily driven by the innate immune cells, namely neutrophils, NK cells and monocytes (fig 1). However, the role of monocytes in driving inflammatory resolution has been largely neglected. We hypothesize that monocytes in BD patients, particularly males, may be altered phenotypically and less effective at propagating resolution in BD.

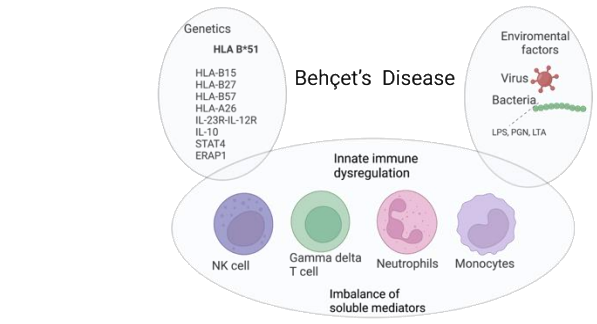

Figure 1. Innate Immune Response in the Pathogenesis of BD

Methods

Isolated monocytes from 49 BS patients and 24 Healthy controls (total monocytes, its subsets (Classical (CM), Intermediate (IM) and non-classical (NCM)) and 2 surface markers (CCR_2 & CX3CR1) were phenotyped by flow cytometry (fig 2). BD monocytes' chemotactic and phagocytic ability to take up pHrodo TM E.coli microbe and Daudi apoptotic cells (efferocytosis, confirmed by immunofluorescence microscopy (fig 3)) were investigated by modified Boyden chamber and flow cytometry, respectively.

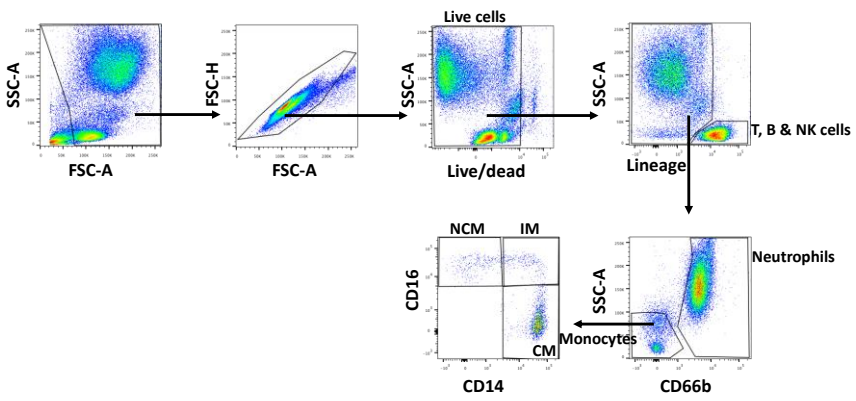

Figure 2. Gating strategy for monocytes and their subpopulation.

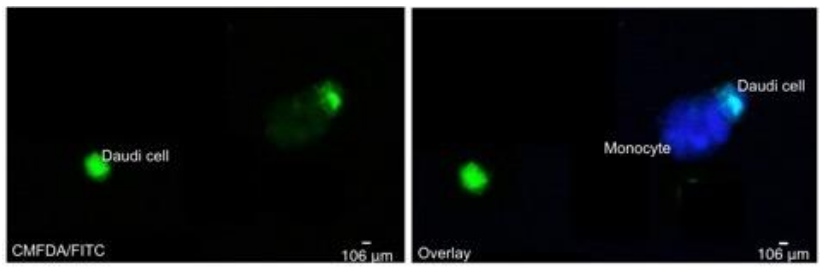

Figure 3. Confirmation of efferocytosis of apoptotic Daudi cells (labelled with CMFDA cell tracker) by monocytes by epifluorescence microscopy.

Results

- Results show reduced expression of chemokine proteins CCR2 and CX3CR1 (Fig 4a & b) on monocytes in male BS patients (fig 4c & d). These are relevant in recruiting monocytes in the inflammatory and resolution phases, respectively.
- Results from the chemotaxis assay show aberrant migration of monocytes in BS patients towards recombinant CCL2 (fig 5a).
- Most importantly, we reported a reduced ability of classical monocytes in BS to phagocytose apoptotic cells (Efferocytosis) effectively (figure 5c).

Results

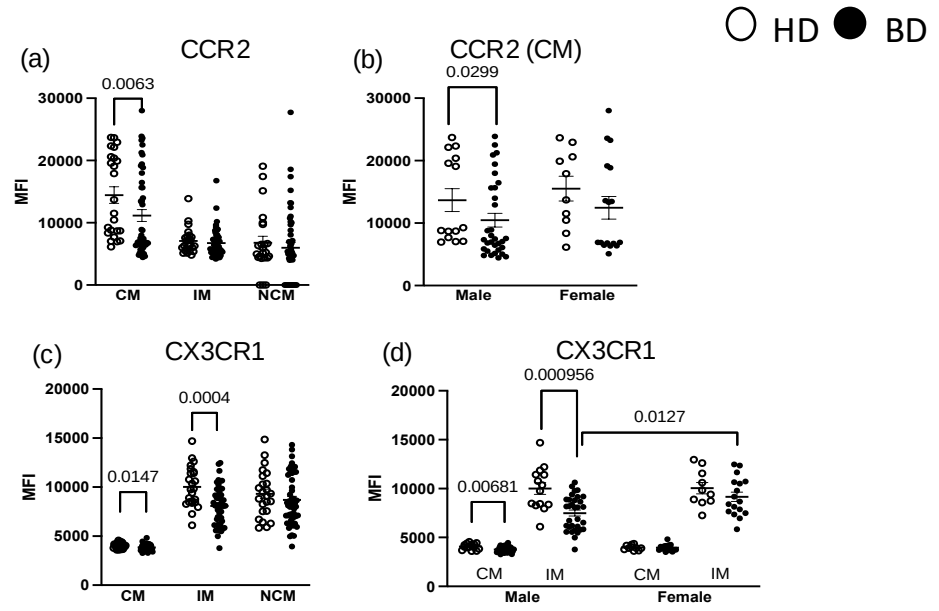

Figure 4. Reduced expression of CCR2 on CM (a) and CX3CR1 on CM and IM (b) in male BD patients (c & d). BD male *n*= 32, BD female *n*=17, C male *n*=14, HC female *n*=10.

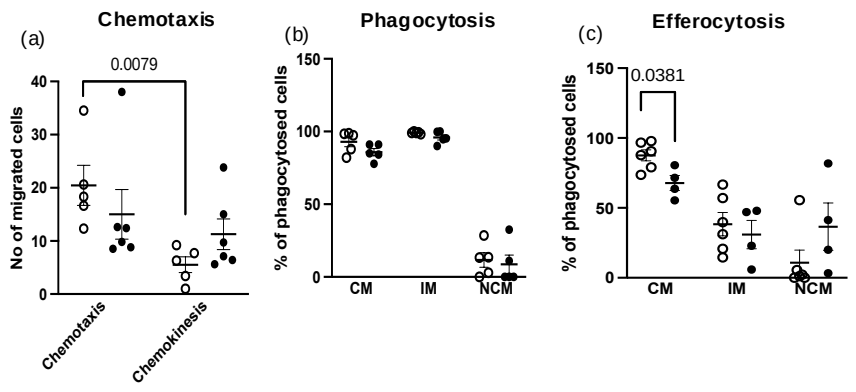

Figure 5. (a) Comparable chemotaxis and chemokinesis in BD patients. (b) Comparable phagocytic ability of BD and HC cohorts to take up microbes. (c) Reduced efferocytosis of apoptotic cells by BD monocytes. Phagocytosis BD &HC *n*=5. Efferocytosis; BD *n*=4 HC *n*=6.

Discussion

- Efferocytosis is a crucial driver of resolution, and our study suggests that monocytes in BS are defective at initiating resolution, especially in male BS patients.
- Further mechanistic investigation of efferocytosis (fig 6) by monocytes may unravel new insights into managing inflammation in BS by harnessing the immune system's ability to dampen inflammation through resolution.

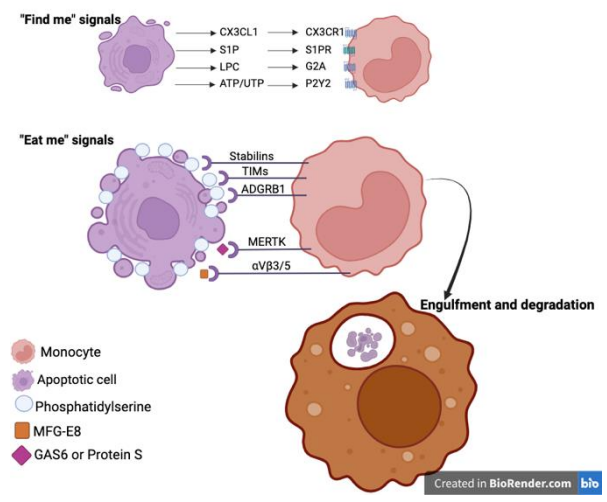

Figure 6- Phases of efferocytosis; understanding the mechanism of efferocytosis in BD.