Scottish Government Health Directorates Chief Scientist Office

CHIEF SCIENTIST OFFICE

FOCUS ON RESEARCH

Researchers
Susan Rhodes, Mhairi Copland, Helen Wheadon

Aim
To identify the underlying mechanisms involved in the inflammatory complications experienced by patients with Polycythaemia vera (PV).

Project Outline/Methodology
PV is characterised by the overproduction of blood especially myeloid blood cells. This occurs due to deregulation of a key signalling pathway which is overactive in 95% of PV patients due to a mutation (JAK2 V617F). This pathway is usually only switched on following growth factor (cytokine) binding, however the genetic change results in cytokine independence and continuous activation of the pathway causing increased blood cell production. Patients with PV experience inflammatory symptoms and have a high risk of developing blood clots, with cardiovascular mortality accounting for a high proportion of deaths. Using JAK2 V617F positive PV patient samples and cell lines this study investigated the molecular and cellular mediators of inflammation. PCR to assess the activity of genes was performed to measure transcriptional regulators and inflammatory interferon response genes. Pro-inflammatory growth factors were quantified in blood plasma samples. Subsets of one type of immune blood cell (monocytes) were characterised by flow cytometry using CD14 and CD16 markers. Monocytes were also matured into macrophages which can be inflammatory (M1) or anti-inflammaroty (M2). The expression of cytokines was then monitored with and without stimulation to determine functionality. The effect of JAK2 inhibition was assessed on JAK2 V617F containing cell lines and primary samples alone and in combination with arsenic trioxide to determine growth (XTT metabolic activity, live cell counts), survival (flow cytometry), gene expression (RT-PCR) and downstream signalling (Immunofluorescence, immunofluorescent co-localisation assay for JAK2 with important inflammation effector proteins SUMO 2/3 and PML & Immunoblotting).

Key Results
The presence of the JAK2 V617F mutation led to signifiant molecular alterations of 32 genes tested in PV samples compared to normal. These include important inflammatory signalling proteins, transcriptional regulators, and interferon response genes. Treatment of cells with JAK2 inhibitors reversed some of these changes. PV patient plasma showed a significant up-regulation of 14/16 pro-inflammatory cytokines examined. This was accompanied by an increase in the number of inflammatory intermediate monocytes. Macrophages generated from PV monocytes also displaying an alteration in cytokine production in response to stimulation. PML is an important regulatory protein induced by inflammatory signals. Assessment of downstream processes indicated that JAK2 localises with both SUMO2/3 and PML, with JAK2 V617F increasing the degree of co-localisation. Treatment with JAK2 inhibitors reduced the level of co-localisation. Treatment of cells led to cell death, with a combination of the JAK2 inhibitor and arsenic trioxide (which causes degradation of PML), enhanced the level of death when compared to single treatment and no drug control.

Conclusions
This study shows an important role of the JAK2 V617F mutation in; transcriptional regulation, immune blood cell function and inflammatory processes. As a result of this PV patients have dysfunction of many mature myeloid blood cells which may explain in part why they experience inflammatory related complications and significant morbidity.

What does this study add to the field?
This study has clarified mechanisms underlying the inflammatory phenotype of PV

Implications for Practice or Policy
These findings can be used to identify more targeted therapies both for disease and symptom control in patients.

Where to next?
The interaction between JAK2 and PML needs further investigation to identify what effect blocking this has on cells. It would also be interesting to assess the effect of JAK2 inhibitors on cytokine levels and monocyte subsets in patients with PV.

Further details from:
Dr Helen Wheadon, Paul O'Gorman Leukaemia Research Centre, 21 Shelley Road, Glasgow, G12 0ZD. Helen.Wheadon@glasgow.ac.uk