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603.LYMPHOID ONCOGENESIS: BASIC

A CX3CR1 (fractalkine receptor) Small Molecular Antagonist (KAND567) Suppressed the Growth Promoting Effect of Monocytes on Chronic Lymphocytic Leukemia Cells

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Background: Chronic lymphocytic leukemia (CLL) is the most prevalent leukemia worldwide. Novel treatment strategies are needed to improve the prognosis. Non-malignant cells of the tumor microenvironment (TME) are of importance for the growth of tumor cells. Monocytes expressing various chemokine receptors as CX3CR1 (the fractalkine receptor (FKNR)) have been shown to promote the growth of tumor cells *in vitro* and *in vivo*. In CLL, monocyte derived nurse-like cells (NLC) support the growth of CLL cells, promoting leukemic cell proliferation and survival by secretion of chemokines/cytokines. In this study, we studied the role of autologous monocytes on the survival of CLL cells and the effects of a small molecule antagonist of CX3CR1 (KAND567/FKNRa). KAND567 is in clinical trials for diseases with an activated fractalkine system as part of the pathobiology including covid-19 infected patients as well as patients with acute myocardial infarction and ovarian carcinoma.
Methods: Primary CLL cells and B cells ($CD19^+$) of healthy donors as well as autologous monocytes ($CD14^+$) from CLL patients (n=22) and healthy control donors (n=11) were cultured alone or in co-cultures (isolated B cells together with autologous monocytes) with the FKNRa (250 and 1000 nM) for up to 120 h. Apoptosis of $CD19^+$ cells and monocytes was analysed by Annexin V/PI staining, Western blotting and the IncuCyte® Live Cell Imaging System. The NBT assay was also applied to check morphological changes of monocytes. Plasma concentration of fractalkine (CX3CL1) was determined by ELISA.
Results and discussion: CLL patients had a significant (p=0.0001) increase in the plasma concentration of CX3CL1 compared to healthy controls. CLL cells as well as normal B cells did not express surface CX3CR1. However, monocytes from both CLL patients and healthy donors expressed high levels of surface CX3CR1. A significantly higher proportion of pro-inflammatory intermediate ($CD14^+/CD16^+$) and non-classical ($CD14^-/CD16^+$) monocytes ($p<0.0001$ and <0.01 resp.) as well as intermediate and non-classical $CX3CR1^+$ monocytes were observed in patients with active CLL compared to healthy donors ($p<0.0001$ and <0.01 resp.). Moreover, $CX3CR1^+$ intermediate and non-classical monocytes were significantly higher in CLL patients with active disease compared to those with early-stage disease ($p<0.0001$ and <0.001 resp.).
A growth supportive effect of autologous monocytes was observed on survival of both CLL cells and healthy donor B cells in 120 h cell co-culture experiments ($p<0.001$ and <0.01 resp.). FKNRa reduced the number of alive CLL cells in a dose-dependent manner in co-cultures with autologous monocytes ($p<0.05$). No effect was seen on $CD19^+$ B cells of healthy donors co-cultured with autologous monocytes. FKNRa did not induce apoptosis of CLL cells alone. Furthermore, FKNRa also inhibited transformation of CLL monocytes to NLC ($p<0.05$) which was not the case for normal monocytes.
Conclusion: The CX3CR1/CX3CL1 (fractalkine receptor/ligand)) axis appears to be activated in CLLand might be of importance for the NLC-driven growth of CLL cells. This is the first report of a small molecule antagonist of CX3CR1 reducing the growth supportive effect of autologous CLL monocytes on leukemic CLL cells. Disruption of growth promotion induced by monocytes may prevent leukemic cell proliferation and survival. Inhibition of autologous monocytes in the TME might represent a new therapeutic strategy in CLL, complementary to treatment with drugs that directly target the tumor cells.

Disclosures No relevant conflicts of interest to declare.

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