

CEACAM1 expression in HL60 human acute promyelocytic leukemia cells under differentiation into neutrophil.

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The neutrophil has a short half-life that is extended by pro-inflammatory cytokines and bacterial products. Normal resolution of inflammation involves the removal of neutrophils and other inflammatory cells by the induction of apoptosis. Disregulation of apoptosis may lead to the persistence of immune cells at inflammatory sites and the development of chronic inflammatory disease. Perturbation of neutrophil apoptosis has been proposed to contribute significantly to tissue damage associated with inflammatory diseases. Novel anti-inflammatory therapies based on the restoration of neutrophil apoptosis have considerable promise, but to identify realistic targets it is important to first understand the precise pathways that regulate apoptosis in neutrophils, then identify survival factors for neutrophils at inflammatory sites and determine their mode of action. CEACAM1 (CD66a), an abundant cell surface protein on neutrophils and shown as a key molecule for apoptosis, is expressed on retinoic acid-induced HL60 acute leukemia cells that differentiate into neutrophils. Our goal is to study neutrophil apoptosis mediated by the CEACAM1 molecule in autoimmune diseases.

The differentiation of HL60 cells into neutrophils with retinoic acid is a well-characterized experimental model for neutrophil apoptosis research. Retinoic acid-induced HL60 cells were not able to enter into S phase, so G₀/G₁ cells were accumulated. After 2 days the cells stopped dividing with apoptosis observed after 4-5 days. CEACAM1 3 or 4-L isoforms were predominantly induced, though almost all isoforms were observed during differentiation. At the same time CEACAM6 was downregulated and CEACAM3 was induced, both of which were expressed in a cytosolic fraction. It is noteworthy that CEACAM3 expression is unique to neutrophils and confirms the neutrophilic lineage of these cells. CEACAM1 positive cells were distinguished into 2 groups depending on the CEACAM1 expression level by FACS analysis. CEACAM1 strongly positive cells didn't enter apoptosis but CEACAM1 weak cells were highly apoptotic. Once cells were stimulated with retinoic acid, they were able to survive much longer if retinoic acid was removed at day 2. Furthermore, continuous treatment with retinoic acid was not required for a strong induction of CEACAM1.

Retinoic acid induced cytokines/chemokines secretion in HL60 cells. IL1 β , MIP1 β , and MCP1 were remarkably increased in the culture media at day 2 compare to the control HL60 cells, however these were significantly decreased by the day 4. We speculated that these cytokine/chemokines signaling pathways are involved in the CEACAM1 induction mechanism.