The Effect of Endothelial Monocyte-Activating Polypeptide II on the Capacity of Dendritic Cells to Attract T-cells

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Abstract

Dendritic cells (DC) are the most professional antigen-presenting cells in the human body and play a central role in adaptive and innate immune responses. These cells encounter endogenous pathogenic microorganisms that can control the way that T cell responses are generated. Endothelial monocyte-activating polypeptide II (EMAP II) is a cytokine released by cells undergoing programmed cell death and some tumor cells and has been shown to modulate endothelial cells as well as immune cells. Our lab has previously shown that EMAP II is capable of attracting dendritic cells. Our hypothesis for this study is that EMAP II will alter the capacity of DC to interact with T cells. We examined this by determining whether supernatant from cells of the JAWS II dendritic cell line cultured in the presence or absence of EMAP II attract cells of the JURKAT T cell line in vitro using a migration assay that assesses the movement of cells through a filter. The results demonstrated that supernatant from EMAP II treated DC attracted more T cells than supernatant from control DC. We concluded that EMAP II stimulates DC to release one or more soluble factors that attract T cells. This suggests that EMAP II released from dying cells might help to stimulate immune responses against infectious agents and cancer but might increase the chances for autoimmune responses following tissue damage that could result in autoimmune diseases such as Lupus and Rheumatoid Arthritis.

Introduction

Endothelial-monocyte activating peptide II (EMAP II) is released from dying cells and attracts immune cells. EMAP II is chemotactic for endothelial cell precursors, neutrophils, and monocytes. Previous studies from this lab have shown that EAMP II attracts dendritic cells (DC). Our hypothesis is that this factor not only attracts DC to sites of skin damage but also modulates their capacity to interact with T cells. DC play an important role in initiating adaptive immune responses by presenting antigens to thymus derived lymphocytes (T cells). In this study, we examined the effect of EMAP II on the capacity of dendritic cells to attract T cells.

Methods

The chemotaxis of DC to T-cells was tested through a T-cell migration assay. This assay consisted of one control (serum free medium) and two variable wells (supernatant from control JAWS II and supernatant from EMAP II treated JAWS II). JAWS II cells were grown at $1x10^6$ cells/ml in the presence of RPMI medium without serum or 0.5 µg/ml recombinant EMAP II for 24 hours at 37°C. After 24 hours the cells were washed to remove the EMAP II and incubated for 24 additional hours. Following incubation, the cells were centrifuged and the supernatant was collected. Jurkat T cells were seeded at a concentration of $1x10^6$ cells/ml in 100 µl in transwell inserts (5 µm pore size) and the supernatant from JAWS II cells, that acted as a chemoattractant, was loaded in the bottom chamber. T cells were allowed to migrate for four hours at 37°C. All of the cells that migrated to the bottom well were collected, centrifuged, and enumerated using a hemacytometer and a light microscope.

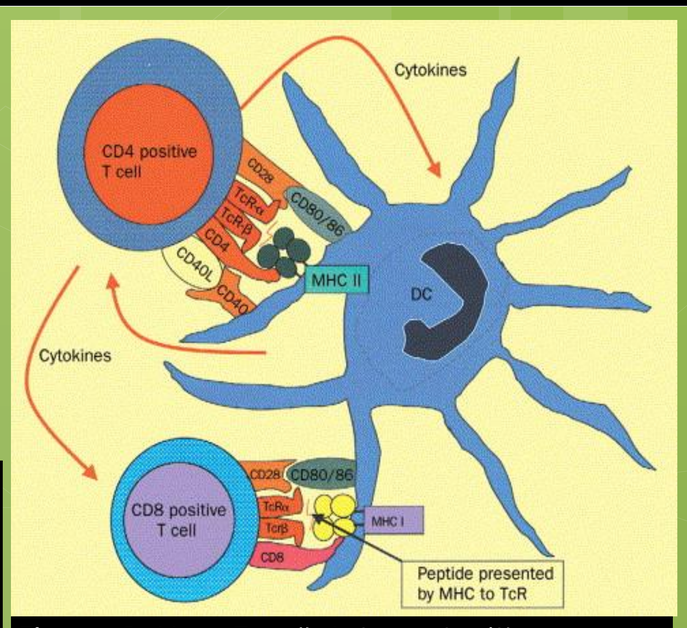

Figure 1: How T cells Interact with Dendritic Cells.

Impact of EMAP II on DC and T cell Interaction

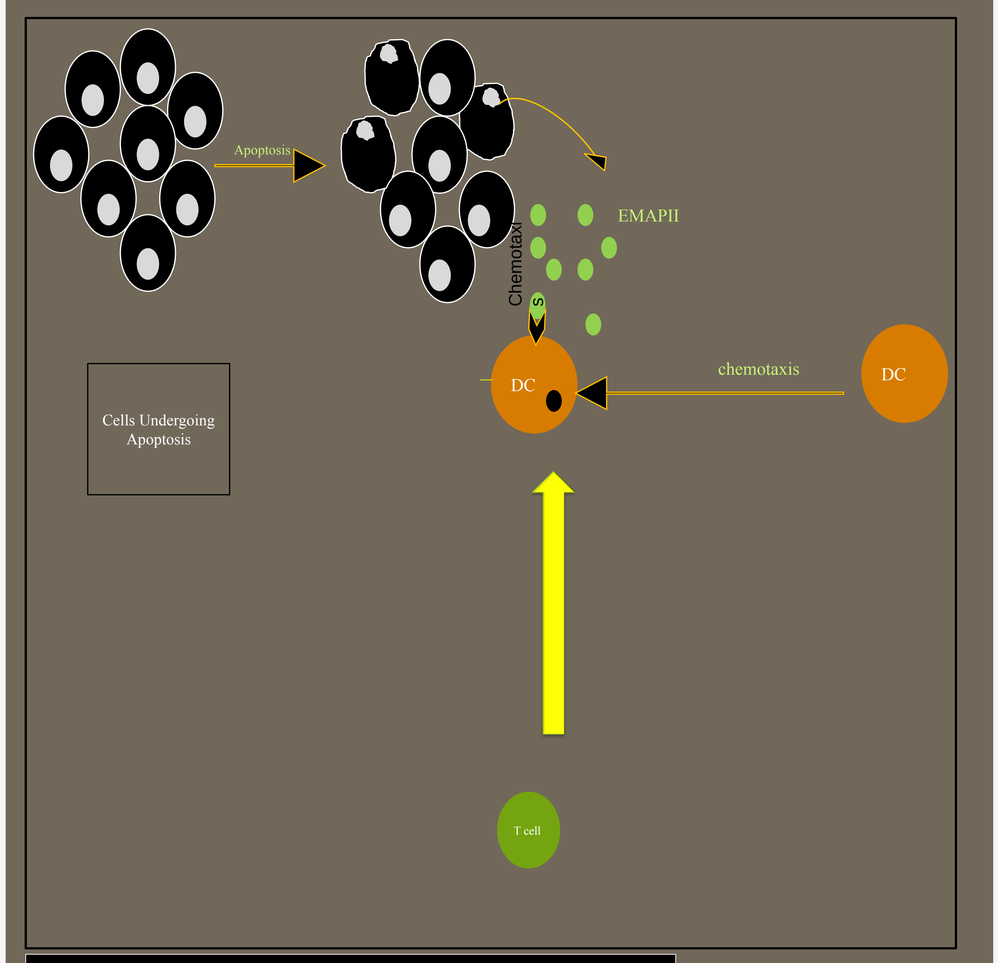

Figure 2: EMAP II attracting T cells to DC

Results and Discussion

In order to determine the impact of EMAP II on the capacity of DC to attract T cells, JAWS II DC were cultured in the presence or absence of EMAP II and the resulting supernantants tested for the capacity to attact T cells of the JURKAT cell line. As shown in Figure 2, supernatant from DC cultured in the presence of EMAP II attracted more T cells than that from untreated DC, suggesting that EMAP II stimulates the release of one or more soluble factors that attract T cells. Chemokines are potent chemo attractants for T lymphocytes and play an important role in adaptive immunity. The increased chemotaxis for T cells induced by EMAP II is likely due to the increased release of one or more chemokines. This suggests that T cells will be attracted to sites of cell apoptosis if EMAP II is released. Whether these T cells will be stimulated by DC or induced to become tolerant remains to be seen.

The dendritic cells that home into sites of tissue trauma may have altered functions that likely reflect the effects of EMAP II. If tolerance is induced, this would help to prevent immune responses to newly released/created autoantigens, thereby preventing autoimmune responses. In the case of cancer, it would benefit tumors that release EMAP II since impaired or reduced phagocytosis may render the dendritic cells tolerogenic. If apoptosis were due to an infection, it would be advantageous for the attracted T cells to be activated. Whether different outcomes of this interaction between T cells and DC occur in different situations remains to be seen. In conclusion, EMAP II is a multifunctional cytokine that attracts and increases the capacity of DC to attract T cells. Ideally, the outcome of this interaction would promote adaptive immune responses to infectious agents and cancer while diminishing those that promote autoimmunity.

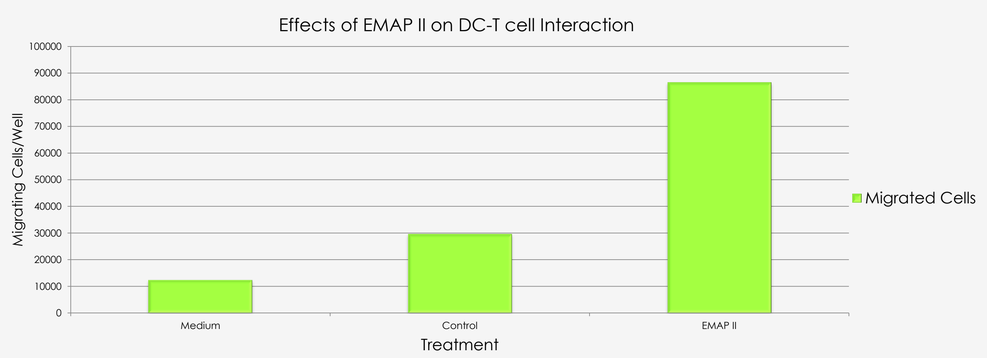

Figure 3: The effect of EMAP II on the capacity of DC to attract T cells