The Effects of Vitamin A on the Migration of Dendritic Cells Towards CCL21.
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Introduction
Dendritic cells (DCs) are antigen-presenting cells that are crucial for the induction of the body's immune response against foreign bodies. DCs respond to specific chemical signals to direct their migration in order to initiate an immune response. In this experiment, we concentrate on chemokine ligand 21 (CCL21) which is one of two ligands for chemokine surface receptor 7 (CCR7) and has been shown to be an essential regulator of DC migration.

Vitamin A derivatives have been shown to inhibit the up-regulation of CCR7, thus interfering with DC migration. Here, we focused on pure Vitamin A as an inhibitor to investigate if it will suppress the chemotaxis of DCs toward CCL21.

Factor VIII (FVIII) is an important coagulation protein. Possible deletions in the FVIII gene may cause a person to suffer from Hemophilia A (HA). Replacement therapy is the current line of therapy used today to control HA.

Problem:
Immune responses initiated by DCs can cause anti-drug antibody compromising safety and efficiency of therapy.

Purpose:
To find an inhibitor to interfere with DC migration in order to modulate the body’s immune response against FVIII.

Hypothesis
Vitamin A will act as an inhibitor and suppress DC migration towards CCL21.

Methods

Murine Bone Marrow Isolation
- The femurs and tibiae were collected from HA mice 8-12 weeks of age.
- Muscle tissue was separated from the bones.
- Bone marrow was flushed out of the bones using PBS filled syringes until all the liquid ran clear.
- The cell suspension was filtered, centrifuged, and resuspended. Cells were then counted using a hemocytometer.

Cell Culture
- Day 0: Isolated DCs were plated in complete media and incubated at 37°C and 5% CO₂ for a total of 10 days.
- Day 3: Fresh media was carefully added without disturbing the basal cell layer.
- Day 6 & 8: Top half layer of media was replaced.
- Day 9: Cultured cells were harvested, collected, and counted.

Chemotaxis Assay
- Cells were plated in Transwell insert plates with DC media, FVIII, and Vitamin A.
- Plate 1 contained CCL21 in its lower compartment while Plate 2 acted as the control with no chemoattractant.
- Plates were incubated for 48 hours after which time cells were counted to determine migration percentage.

Results
- Plate 1, treated with CCL21, had greater migration activity than Plate 2, without CCL21.
- Cells treated with FVIII + Vitamin A, showed significantly greater DC migration compared to the other groups.

Conclusions
- Contrary to our hypothesis, Vitamin A failed to act as an inhibitor and suppress DC migration.
- According to our results, Vitamin A was shown to induce the actual migration of DCs toward CCL21.
- Our findings suggest that Vitamin A may play a larger role in the DC ability to migrate towards a chemoattractant than we had anticipated.

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References