The role of dendritic cell subsets in atopy following skin allergen challenge

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Ομάδα άσθματος
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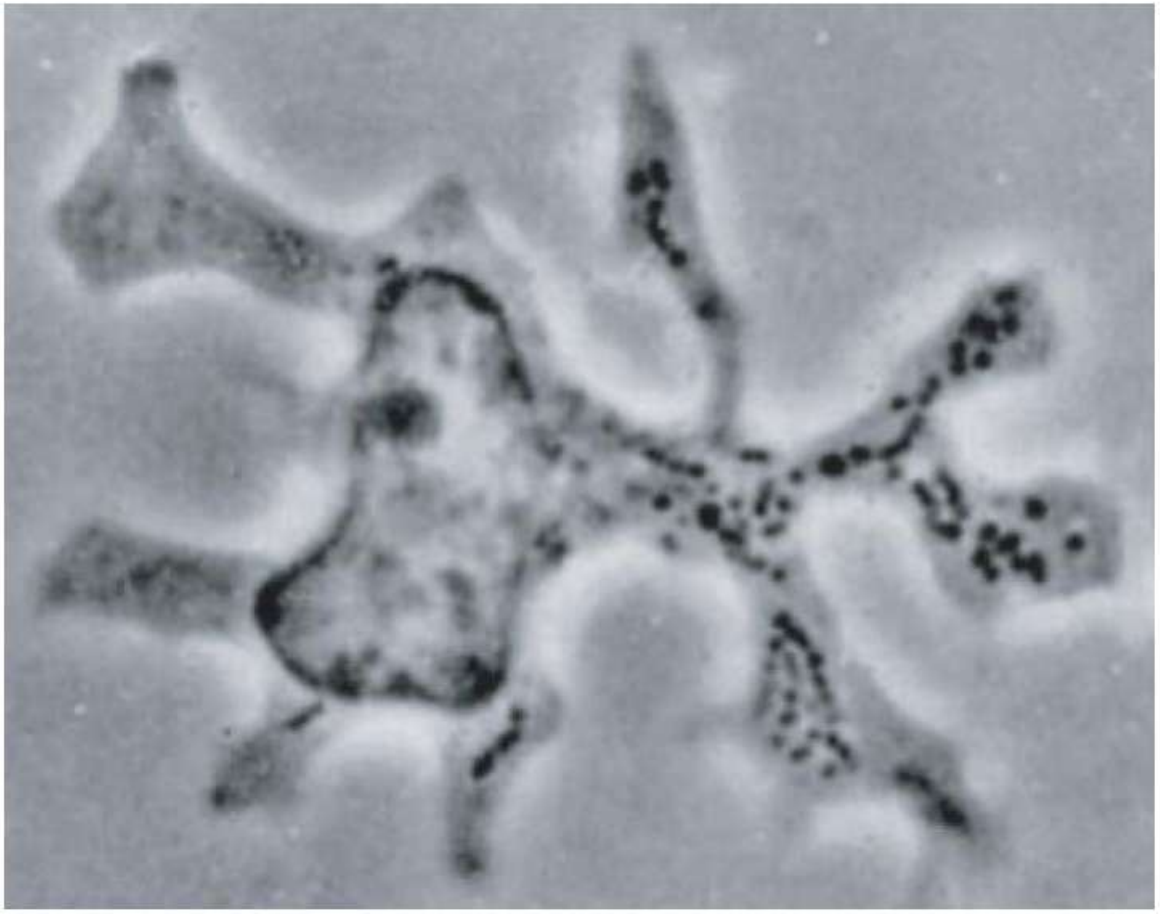
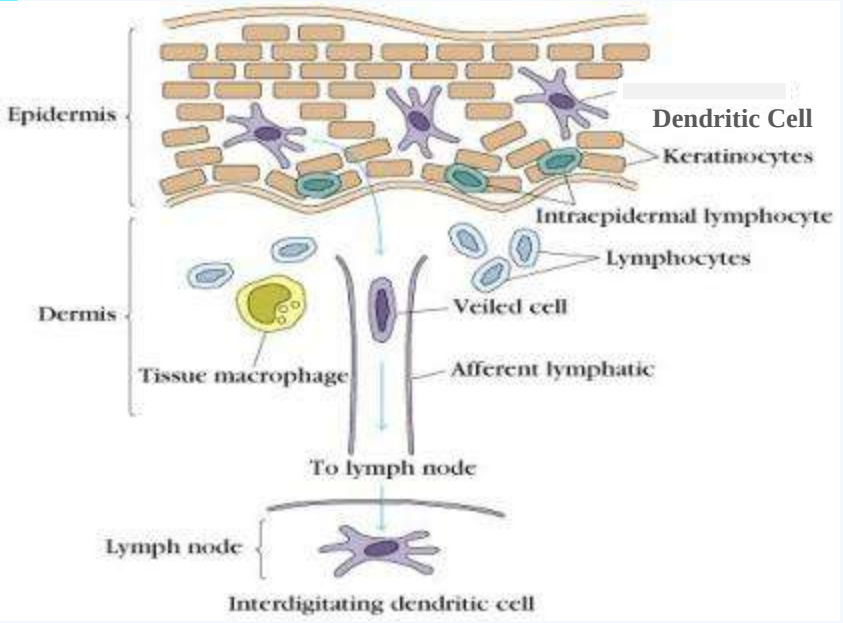
Dendritic Cells



- Infection
- Cancer
- Transplantation
- Autoimmunity and chronic inflammation
- Allergy
- Vaccines

Ralph M. Steinman, Nature 2007

Dendritic Cells



- Form a network of cells in the upper layers of epithelium of the airways, and the skin.
- Immature state: recognize, capture and process microbial pathogens
- Mature state: migrate to the draining lymph nodes => present pathogenic antigens and activate the adaptive immune system

Major Antigen Presenting Cells - link innate to adaptive immune responses

conventional (cDC) – ($CD11c^+BDCA1^+$) ⟹ enhance immunity [1]
plasmacytoid (pDC) – ($CD123^+BDCA2^+$) ⟹ suppress immunity [1]

During allergic responses ! (in mouse models)

The balance (number, kinetics, proportion) between cDC and pDC subsets regulates immune responses and allergic inflammation

Which is the role of each DC subset during acute and chronic phases of allergic inflammation in humans?

A model of acute and chronic allergic inflammation which involves several allergen challenges in the skin of humans with atopy.

[1]*Xanthou et al, Nat Med 2006/ Hammad et al, J ACI 2006./ Lambrecht et al JCI2000, / de Heer et al, J Exp Med 2004*
[2]*Zaba et al, J Inv Derm 2008*

Aims of the study

1. To identify the kinetics of dendritic cell subsets (cDCs and pDCs) in the peripheral blood and skin of humans with atopy (atopics) after one and multiple skin allergen challenges. *Are they recruited at the inflammed skin and at which time point?*

2. To analyze the phenotype of dendritic cells (**plasmacytoid DC, cDCs,, Langerhans cells**) in the skin of a) healthy volunteers, b) atopics and, c) atopics following one or multiple skin allergen challenges.

3. To correlate DC subset recruitment at the skin with the expression of relevant inflammatory markers (i.e. osteopontin).

4. To analyze dendritic cell functional elements (i.e. their maturation state, CD40, CD80, CD83, CD208, CD205) in the skin.

Skin allergen challenge model: A suitable model for the study of allergic inflammation.

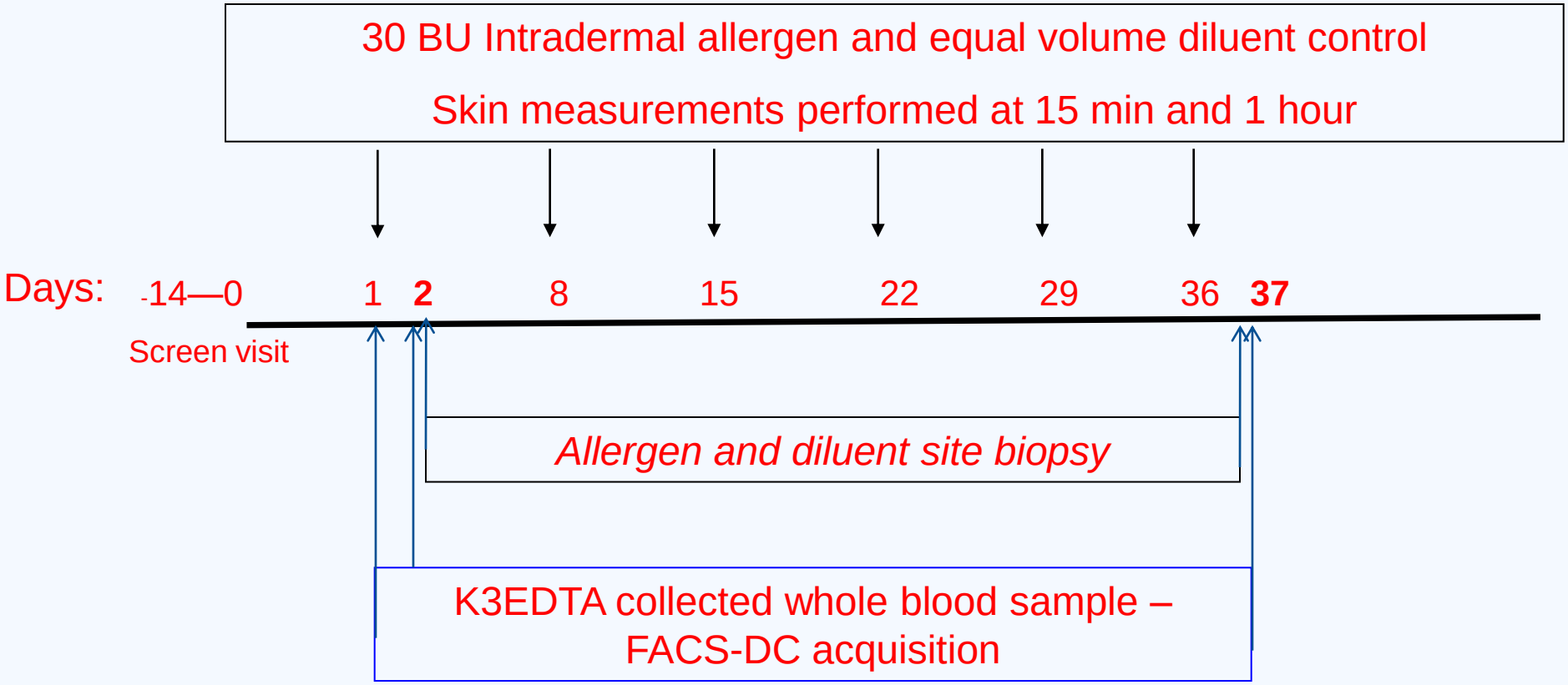
- Easily accessible tissue
- Intradermal challenges are repeatable -an exact dose of allergen is easily administered
- Repeated allergen challenge in the skin imitates the chronicity of allergen exposure in a standardized fashion [1]
- Much less invasive and safer human model than other allergic models (i.e. repeated bronchial challenges and bronchoscopic biopsies in asthma)

Can be used as a study model of chronic allergic inflammation in diseases such as asthma and allergic rhinitis [1]

[1] *Bruin-Weller MS et al,Clin Exp Allergy 1999.*

Study Design

- 10 Atopic Patients- positive skin prick test to one or more allergens (informed consent)
- 6 weekly intradermal challenges of allergen and diluent
- Skin biopsies at diluent and allergen site 24h after first and 6th challenge
- Whole blood sample for FACS acquisition before,1h after 1st challenge and at the time of biopsies



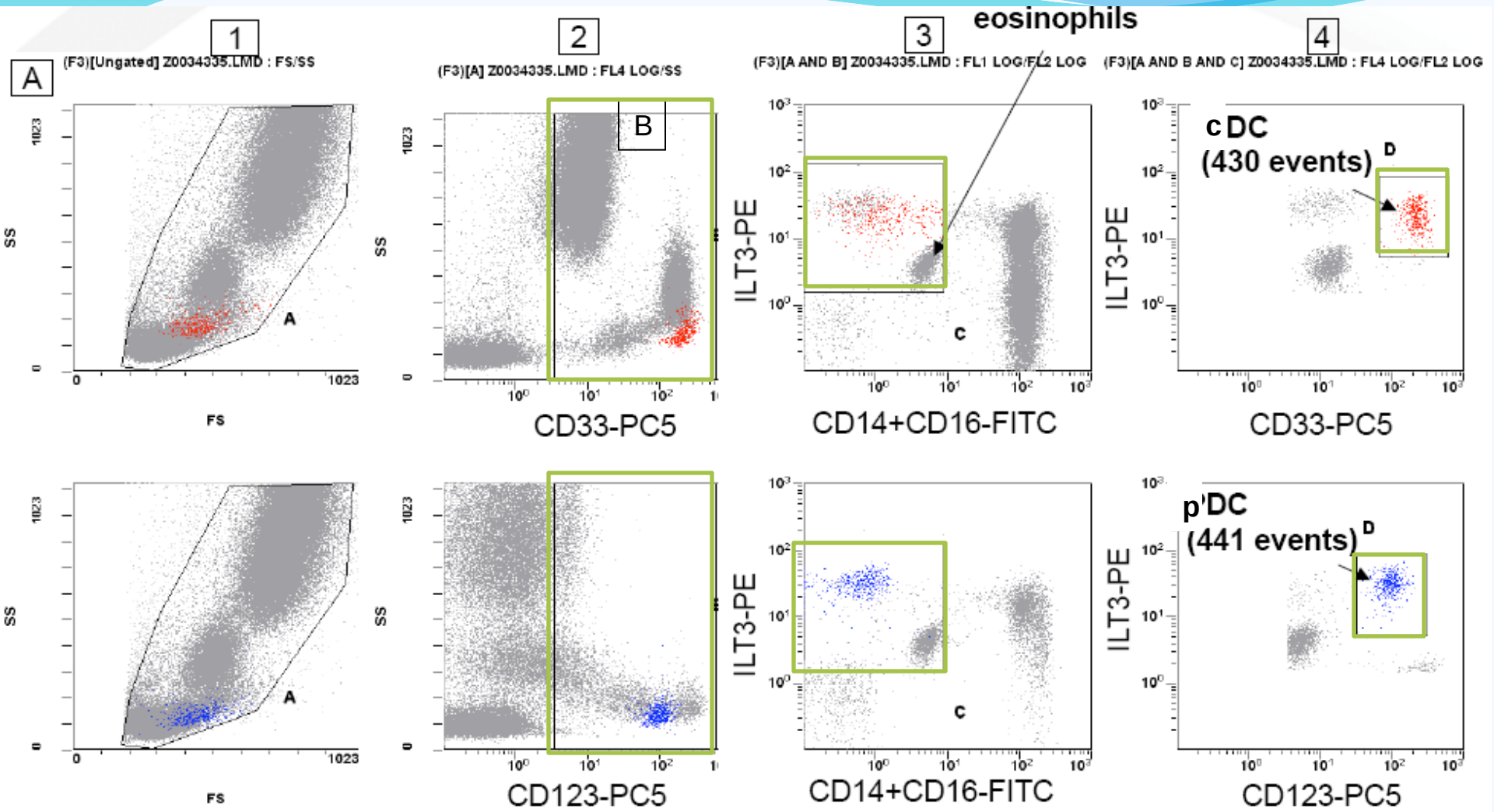
Flow Cytometry

Flow cytometric analysis of peripheral blood dendritic cell subsets

pDCs: CD(14+16)-FITC $^{-}$ / CD85k (ILT3)-PE^{+} / CD123-PC5^{+}
cDCs: CD(14+16)-FITC^{-} / CD85k (ILT3)-PE $^{+}$/ CD33-PC5^{+}

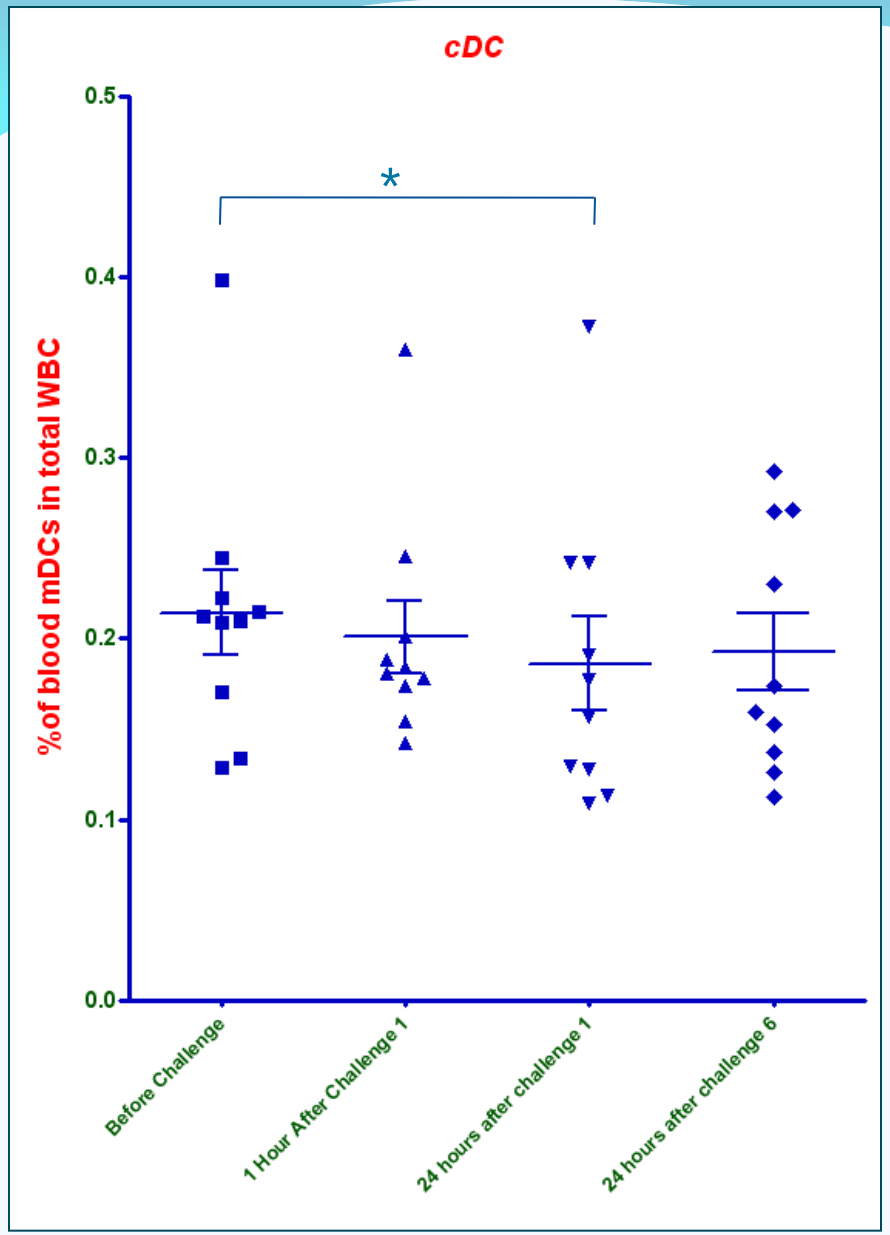
- positive selection of all CD123^{+} or CD33^{+} cells
- restriction to monocytes and DCs (ILT3^{+}), and exclusion of all basophils (ILT3^{-}) with ILT3
- exclusion of all monocytes, with CD14+CD16

Flow Cytometry



Flow cytometric data using blood from a normal volunteer. For cDC and pDC identification, a 4-step analysis is performed.

1. In histogram 1 all leucocytes are gated while eliminating debris in an A region.
2. In histogram 2 (PC5 vs SSC), gated on A, a B region is created around all PC5 positive events (CD33 for cDC identification, or CD123 for pDC identification).
3. Histogram 3 (FITC vs PE) is gated on A AND B. C region is created to include all FITC dim to negative events, and all PE positive events (eosinophils are used as a help forpositionning region C).
4. Histogram 4 (PC5 vs PE) gated on A AND B AND C enables identification of cDC or pDC in the D region.



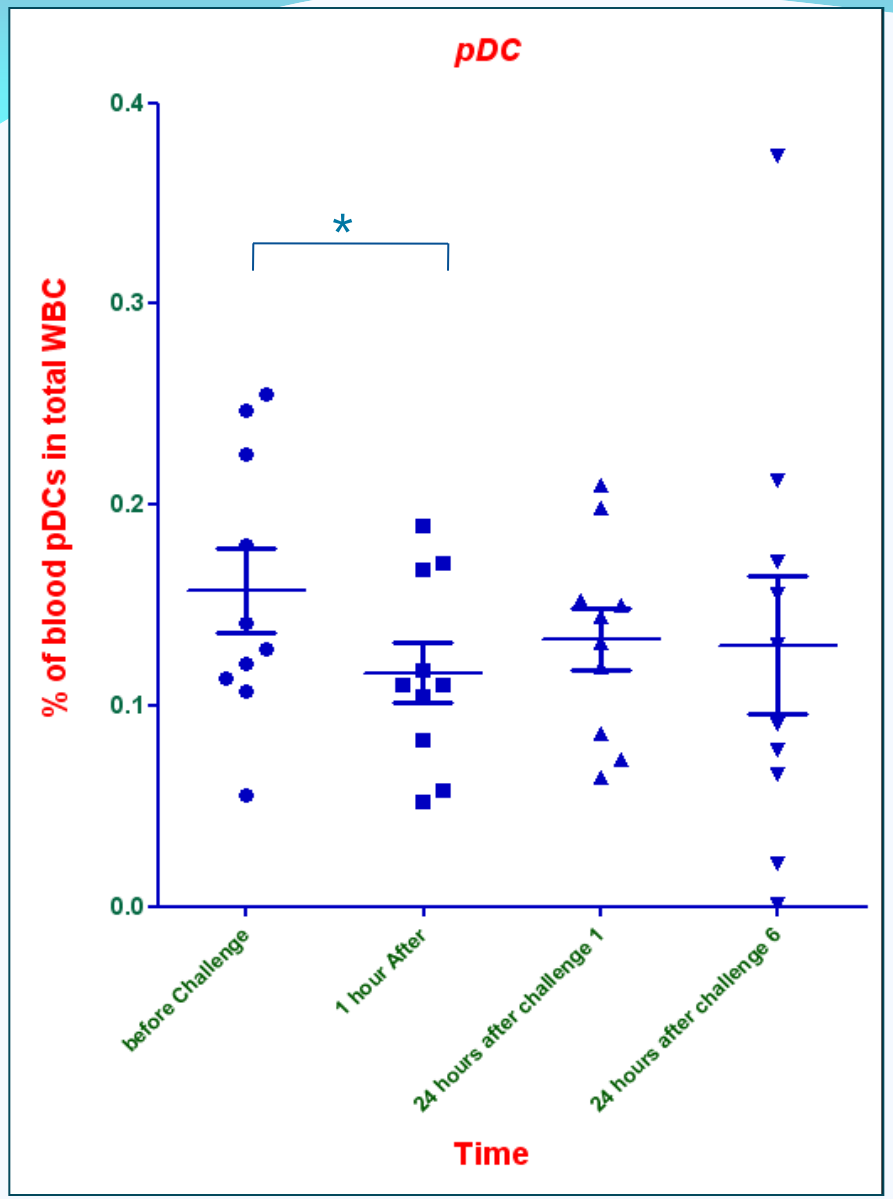
Kinetics of cDCs in peripheral blood

cDC numbers decreased in the blood 1h post allergen challenge.

cDCs numbers significantly decreased at 24h.

** p < 0,05*

Percentage of blood cDCs in total white blood cells. Data are presented as mean ±SEM.



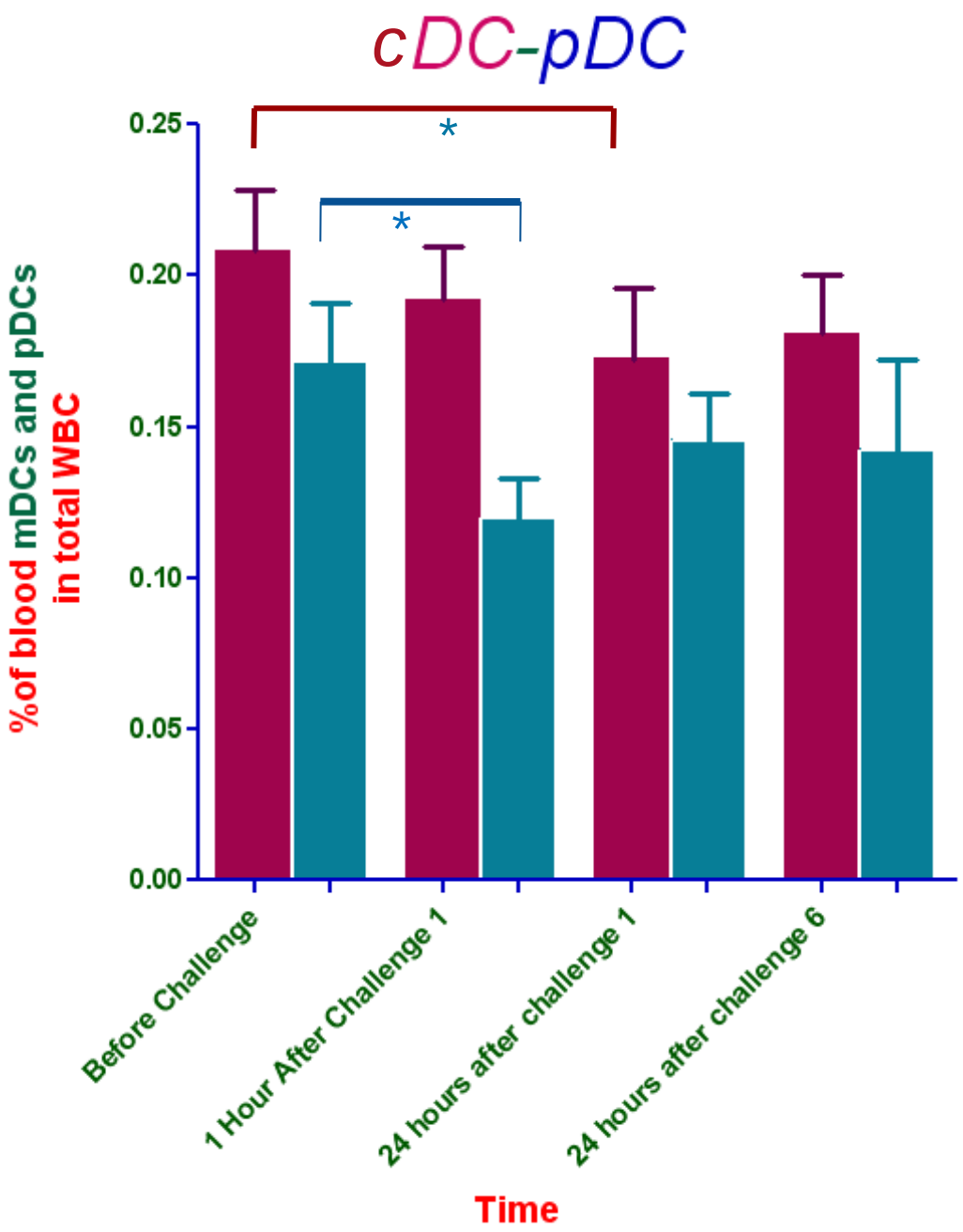
Kinetics of pDCs in peripheral blood

pDC numbers <u>significantly decreased</u> in the blood 1h post challenge

pDC numbers <u>started to increase</u> by 24h.

* p<0,05

Percentage of blood pDCs in total white blood cells. Data are presented as mean ±SEM



There was a fall in cDCs which started 1h post challenge but reached significance at 24h.

This fall was also noted in pDCs which was much quicker as it was significant at 1h, but it was no longer significant by 24h.

This decrease in DC numbers in peripheral blood suggest that they travel to the skin following allergen challenge.

There is a difference in the kinetics of the two DC subsets, suggesting that
1)each plays a different role in allergic inflammation.
2)each subset is regulated differently during allergic inflammation.

Immunofluorescence

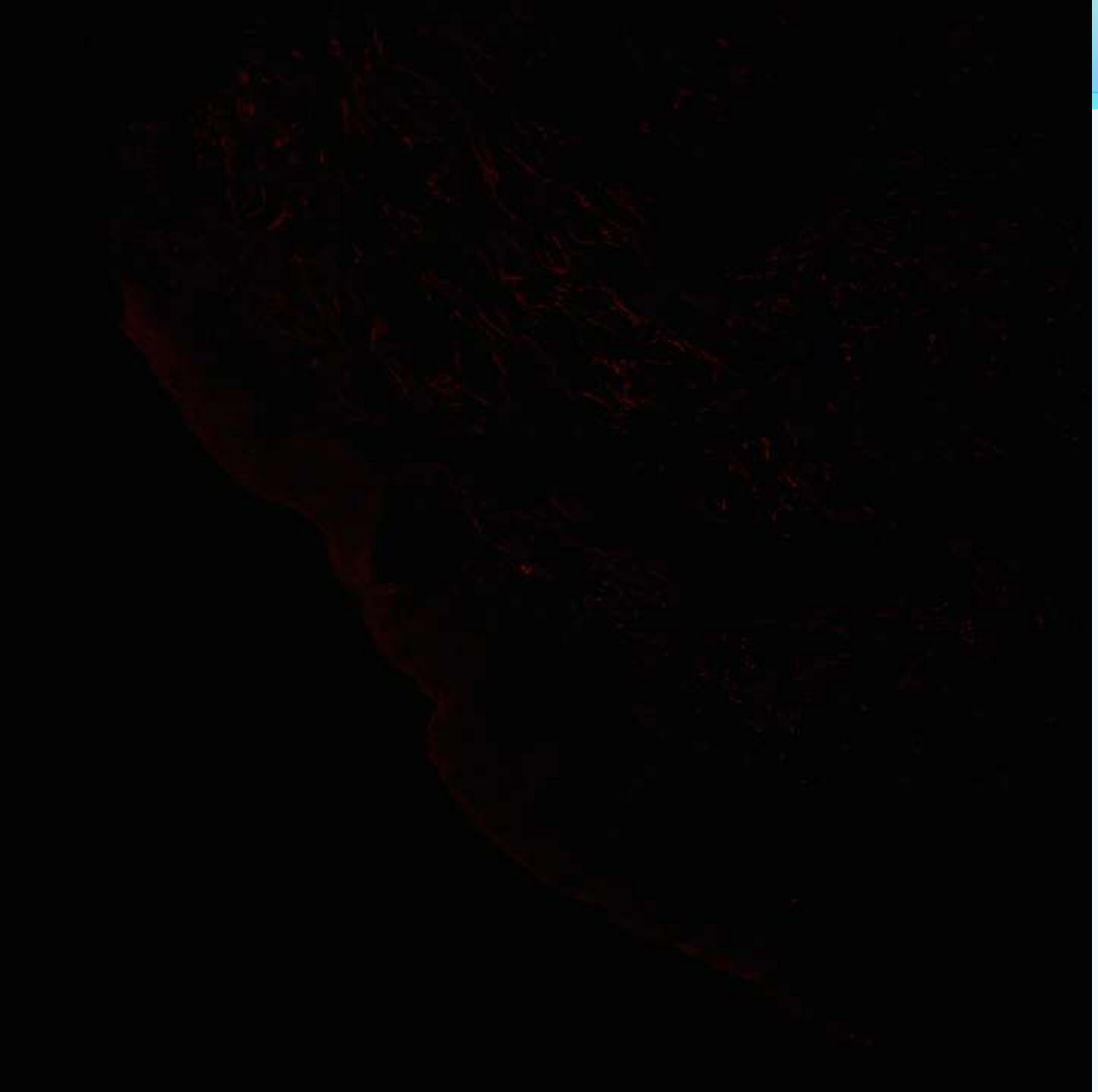
- BDCA-1, CD11c ⟶ cDCs
- BDCA-2 ⟶ pDCs
- HOECST ⟶ nucleus

BDCA-1 +**Alexa Fluor-568**

CD11c + **Alexa Fluor-488**

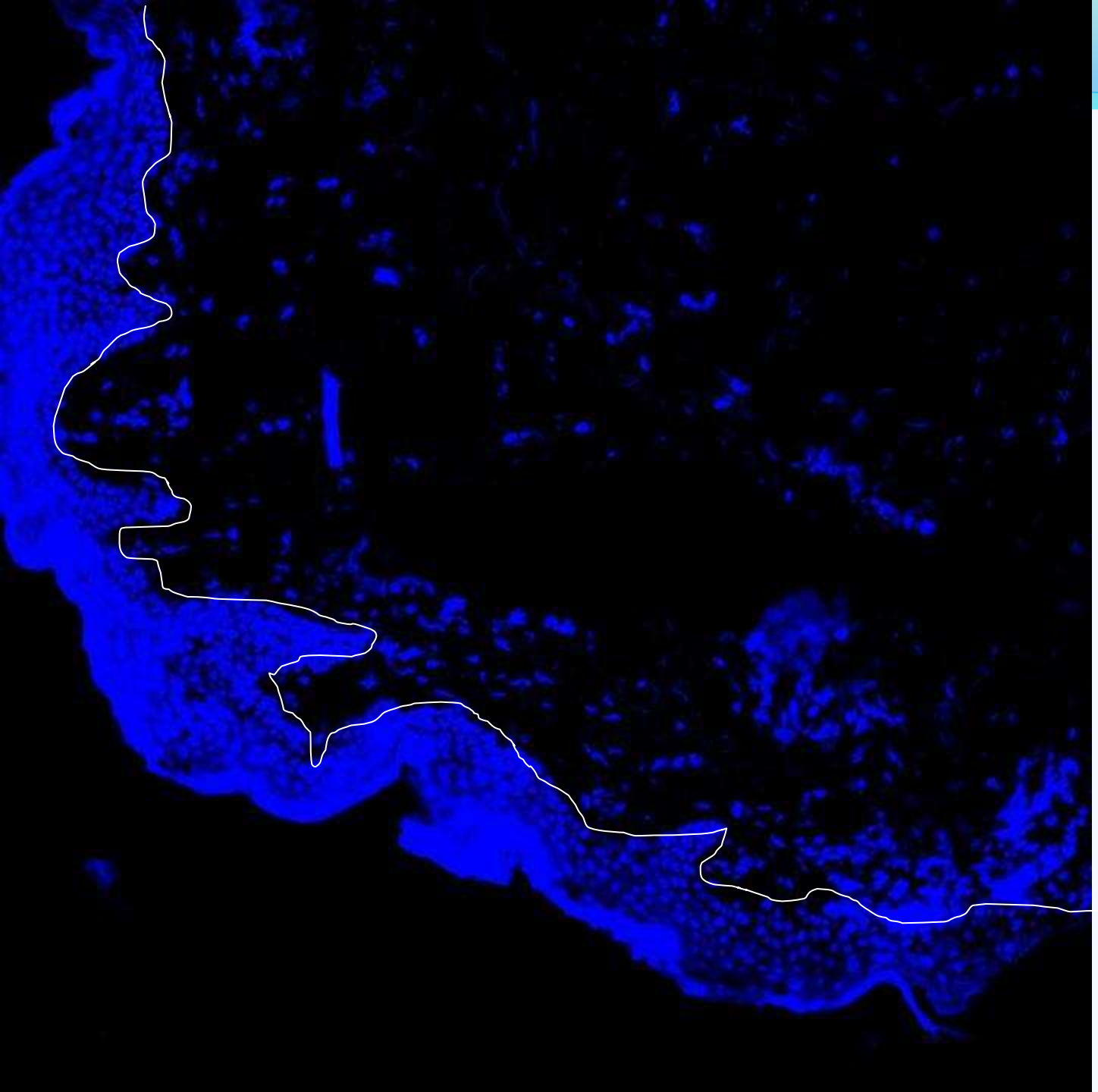
BDCA-2 +**Alexa Fluor-488**

HOECST



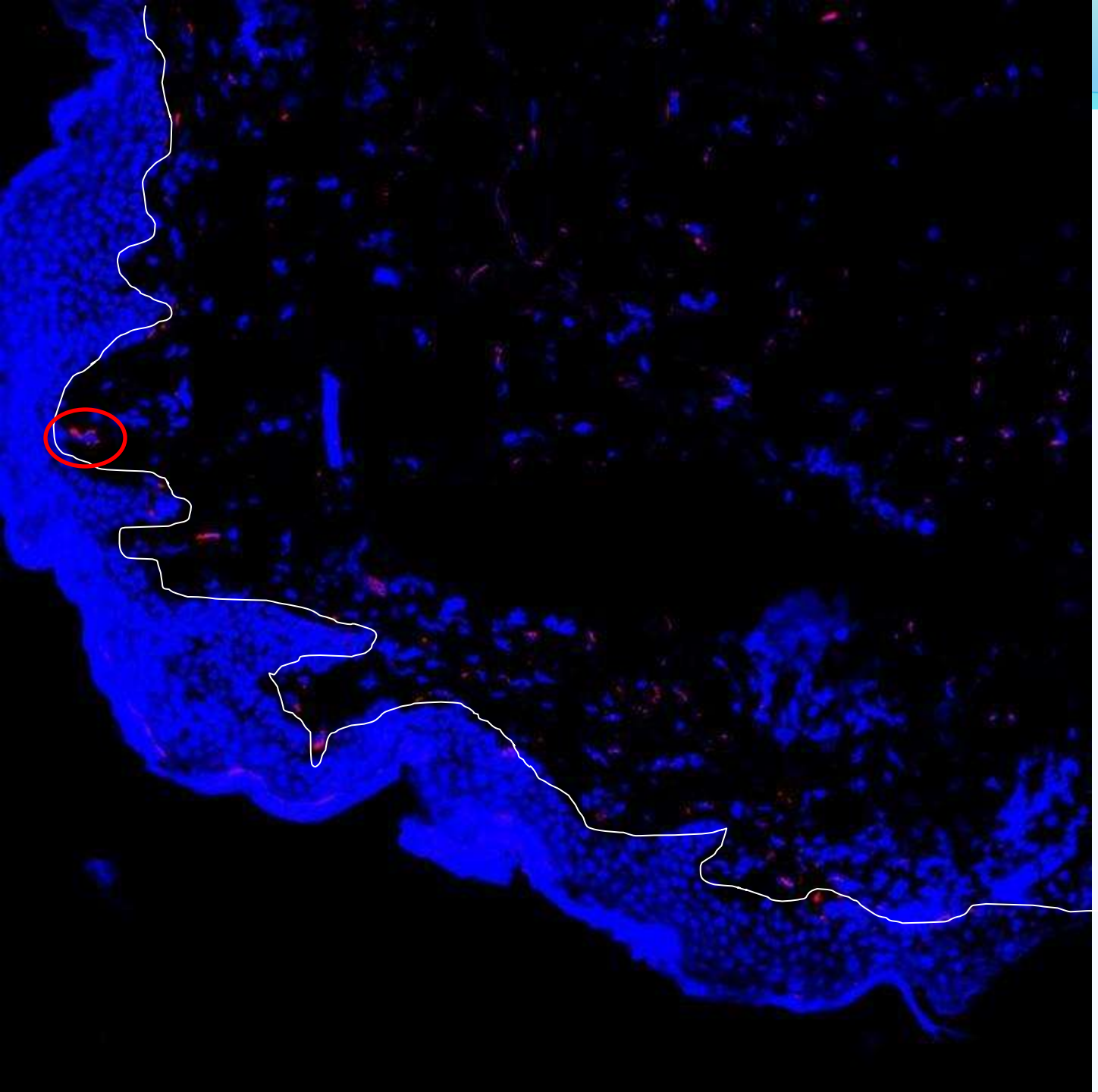
BDCA-1 +
Alexa Fluor-568

Red channel



HOECST

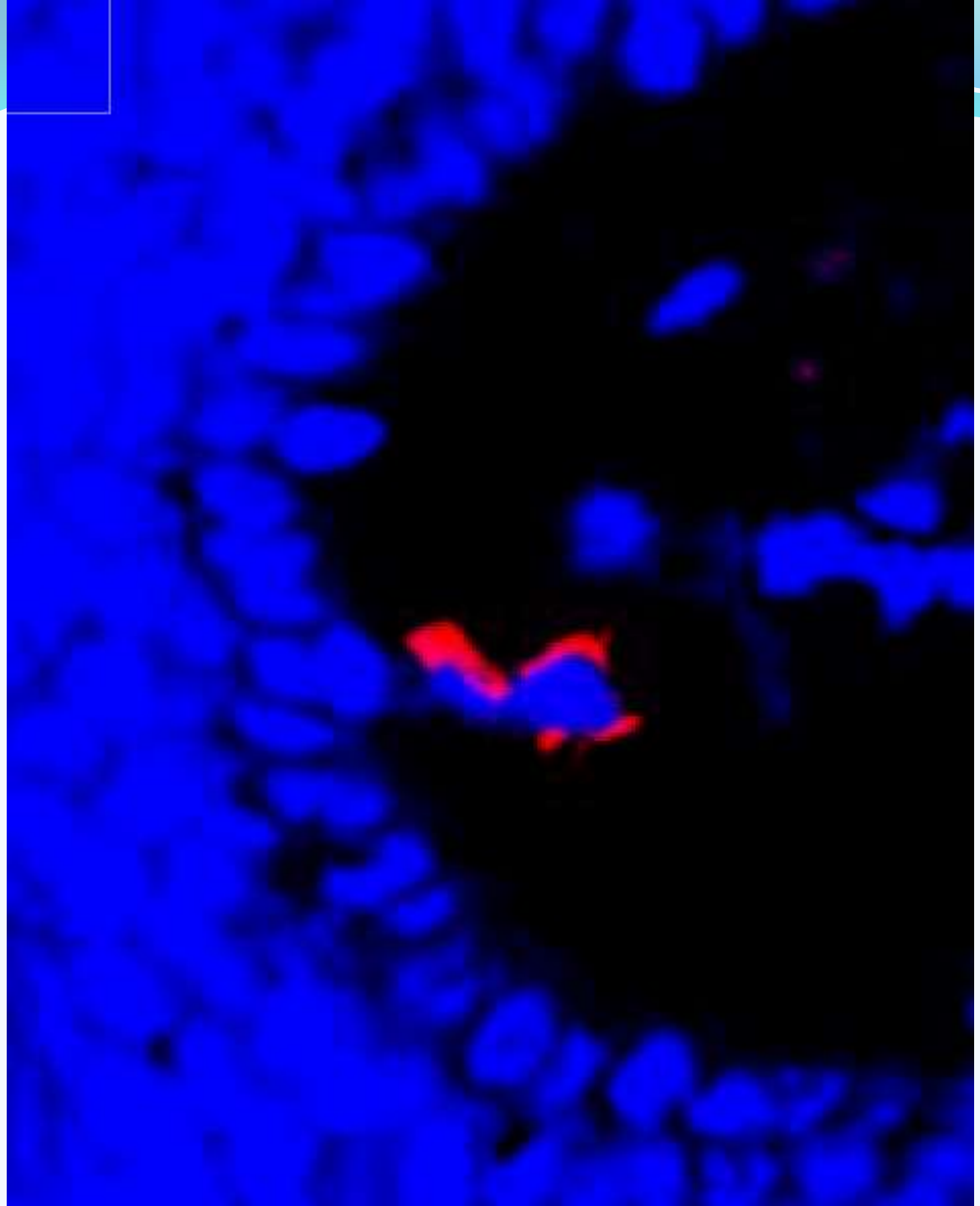
Blue Channel



BDCA-1 +
Alexa Fluor-568

+ HOECST

Combined



Future Goals

1. Completion of immunofluorescence with confocal microscopy to identify the phenotype of DC subsets in skin biopsies of atopics following allergen challenge.

2. Correlation of DC subset recruitment with the expression of osteopontin[1] and activin-A in skin biopsies of atopics following allergen challenge.

Preliminary data: significant increase in Opn expression in skin biopsies following allergen challenge

[1]*Xanthou et al, Nat Med 2006*

Aknowledgments

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Thank you

THE END