Dendritic Cell Research
The ANZAC Research Institute
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Title: The Identification of a Novel CD2 High Plasmacytoid Dendritic Cell Subset in Human Tissues

This project involved identifying novel plasmacytoid dendritic cell (pDC) subsets, one $CD2^{hi}$ and the other $CD2^{lo}$ by flow cytometry, in human bone marrow and lymph nodes. This required the development of a two colour immunohistochemistry (IHC) method. However attempts to label CD2 on pDC by IHC in the past have been unsuccessful. This is most likely because previous studies have identified pDC in the inter-follicular T-cell zones of lymph nodes, where abundant CD2-expressing T cells obscure CD2 on pDC.

Hence we had two challenges: firstly developing a method for the robust identification of pDC within both lymph nodes and de-calcified bone marrow biopsies; secondly developing an innovative method to identify the $CD2^{hi}$ subset. Antibodies to CD123 and TCL-1 were trialled as pDC markers, but were not adequately specific. Polyclonal antibodies for E2-2 and SpiB were purchased (pDC-specific transcription factors), however their specificity was poor over a range of concentrations. Subsequently, a newly available CD303 antibody was purchased and provided excellent specificity in both bone marrow and lymph node samples (Figure 1).

With regards to identifying the $CD2^{hi}$ subset, a breakthrough occurred when we defined this $CD2^{hi}$ subset as having high levels of BCL-2 at both an mRNA and protein level. Increased levels of this anti-apoptotic molecule explained our original observations regarding the survival of this subset in conditions of stress *in vitro* and in glucocorticoid-treated Multiple Myeloma (MM) patients. Subsequently, we have shown that BCL-2 identifies a pDC subset in lymph nodes and bone marrow, with the same frequency as the $CD2^{hi}$ pDC. We believe that BCL-2 is an excellent surrogate marker for this novel immune cell (Figure 1). We have also labelled pDC in the bone marrow with CD2, where it is feasible because of the lower frequency of T cells, and found $CD2^{+}$ and $BCL\text{-}2^{+}$ pDC to be present at the same frequency. With regards to their localisation with malignant plasma cells, a prominent publication in this field has suggested pDC are greatly enriched in the marrow in MM (Chauhan et al. Cancer Cell 2009). Our data suggests this is not the case, with infrequent pDC being found in the BM, and with no clear association with MM cells (Figure 2). This negative data is of vital importance to the field, as international efforts are currently underway to target pDC as a stromal element on the basis of their increased frequency.

This grant in aid has allowed the completion of a vital component of this project has fostered a long-term collaboration with Dr Kenneth Lee at the Department of Anatomical Pathology at Concord Hospital and has contributed to a manuscript which is currently under consideration at The Journal of Immunology. A further manuscript including this data is being prepared. I have attached some representative images of this work should you be interested, and I am more than happy to share additional data.

Regards,

Dr Christian Bryant 26/6/15

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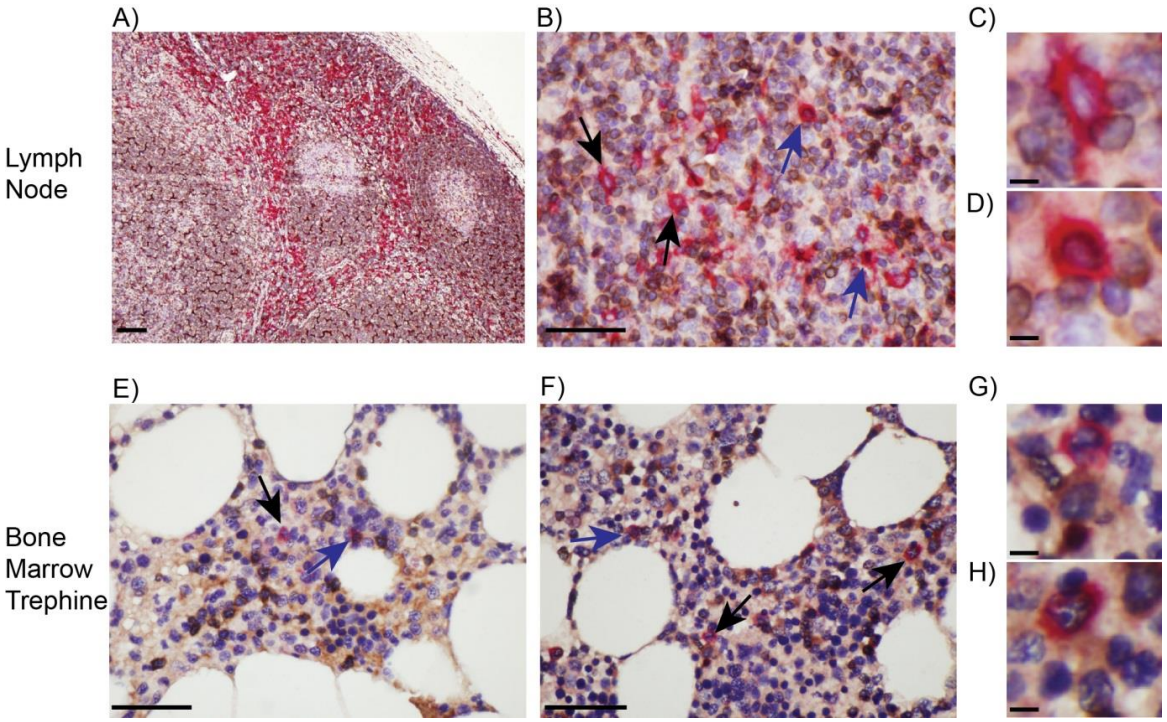

Figure 1. CD2 and BCL-2 labelling identify a pDC subset in human tissues. A) Paraffin embedded reactive lymph nodes contain $CD303^+$ (red) pDC primarily located in the paracortex around high endothelial venules (20x magnification scale bar is 100um). **(B)** pDC can be seen as either single stained $CD303^+$ (red, black arrows) or double stained $CD303^+$ $BCL2^+$ (blue arrows) cells. **(C)** and **(D)** shown high magnification views of single $CD303^+$ or $CD303^+$ $BCL2^+$ cells respectively; (one representative image of 2 donors is shown) Immuno-histochemical analysis of CD303 and either CD2 **(E)** or BCL2 **(F)** in bone marrow trephine specimens from MM patients at diagnosis revealed rare single $CD303^+$ positive (black arrows) and double positive (blue arrows) scattered throughout the bone marrow with high magnification views of $CD303^+$ and $CD303^+$ $BCL2^+$ single cells shown in (G) and (H); n=3; 1 representative donor is shown. B, E and F; magnification is 60x; Scale bar is 100um. C, D, G and H; magnification is 60x; scale bar is 10um. MM, multiple myeloma; Fo, follicle; HEV, high endothelial venule. In all cases CD303 is stained red and both BCL2 and CD2 respectively are stained brown.

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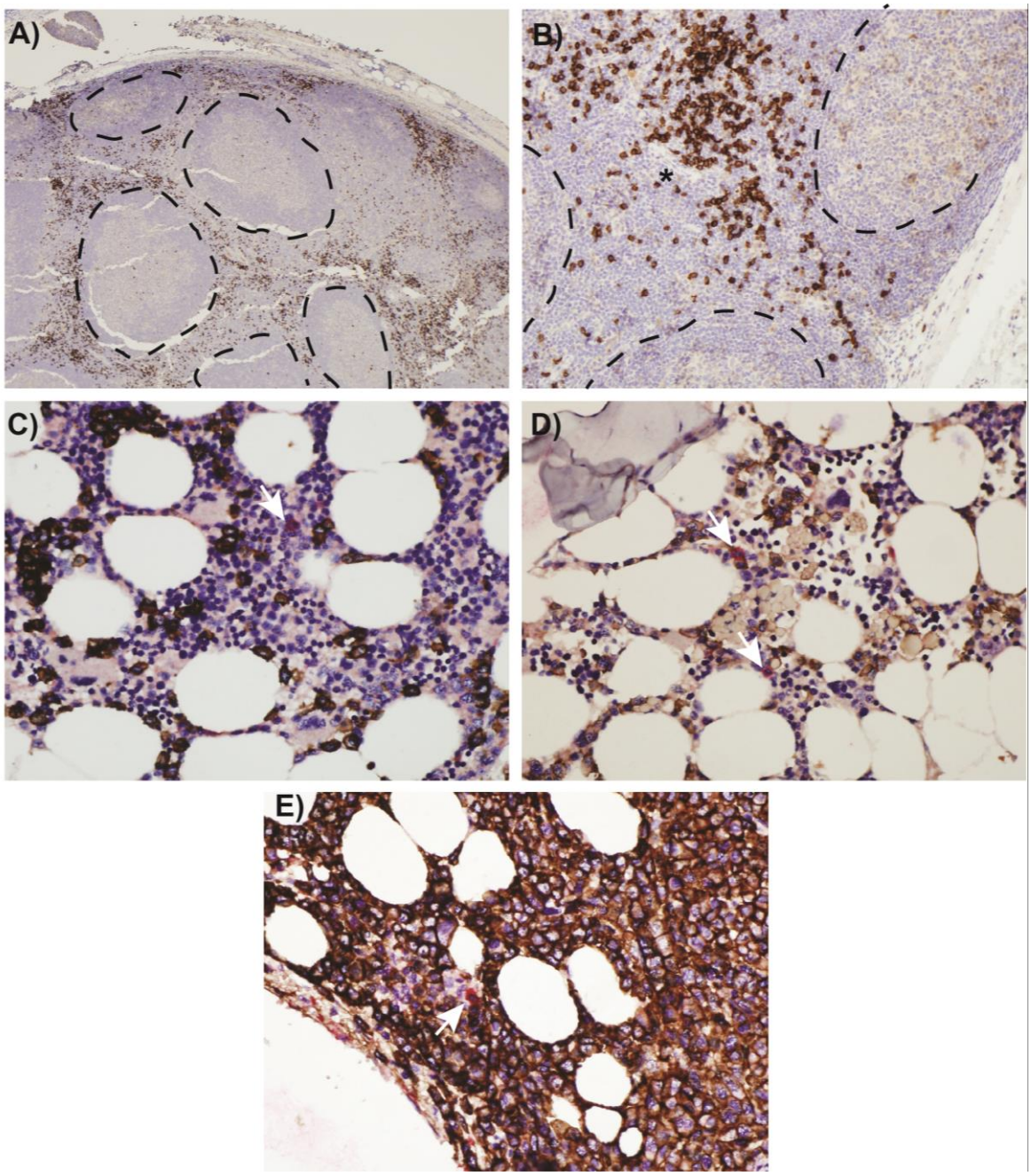

Figure 2. CD303^{+} pDC are infrequent in MM and do not closely associate with plasma cells **(A-B)** Paraffin-embedded reactive lymph node specimens were labelled with CD303 (dark brown). Large numbers of CD303+ pDC can be seen between the follicles (dashed lines) in the interfollicular T cell regions **(A-B)**, clustering around the high endothelial venules (asterix) (B)(Photos of one of 3 representative donors, A at 1.25X and B at 20X magnification). **(C-E)** Paraffin embedded bone marrow biopsies were labelled with CD138 (dark brown) and CD303 (red). Rare scattered pDC could be identified and they did not seem to cluster with the malignant plasma cells (representative photos of 3 MM donors all at x20 magnification).