Cross-priming of the melanoma antigen, Melan-A, by skin dendritic cells

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Introduction

Cutaneous melanoma is the most dangerous and aggressive form of skin cancer, characterised by the transformation and invasive growth of melanocytes\(^1\). The incidence rate of melanoma is rising worldwide\(^2-3\).

Radical excision of the primary tumour is currently the only curative treatment option\(^4\), however surgery is rarely an option for patients with metastatic disease. The prognostic outlook for metastatic disease is bleak, with two-year overall survival rates of 10%\(^5\). Melanoma has poor sensitivity to traditional treatment methods involving chemotherapy and radiation therapy. Furthermore, recently approved immunotherapies vemurafenib and ipilimumab have proven to be of limited, objective clinical use due to resistance and poor response respectively\(^1,6-7\). The latter is partly due to paucity in knowledge about the biological function of the antigen presenting cells (APCs) responsible for processing melanoma-associated antigens and generating a cytotoxic anti-melanoma immune response.

Dendritic cells (DCs) constitute a heterogenous population\(^8\) of professional APCs, with a pivotal role in linking the innate and adaptive immune system. DCs are found in peripheral tissues such as the skin, where they sample the local microenvironment and migrate to the draining lymph nodes in order to interact with T cells and initiate immune responses. DCs harbour the unique capacity to induce \textit{de novo} antigen-specific cytotoxic T lymphocyte (CTL) responses by the phenomenon of cross-priming, see Figure 1.
The human immune system is capable of mounting a cytotoxic response against melanoma\(^9\), and thus enhancing specific tumour-killing immune responses against melanoma presents as an attractive therapeutic strategy. To date, several melanoma-associated antigens have been identified and the strikingly immunodominant\(^10\), well characterised melanoma-associated antigen, Melan-A, has generated considerable clinical interest with regards to developing an anti-melanoma vaccine\(^11\).

As critical regulators of immune responses, DCs are of significant importance in designing rational anti-cancer vaccines\(^12\)-\(^13\). Very little is known about how human skin DCs process cancer antigens to generate tumour killing responses and whether this can be clinically translated to advance cancer therapy. Demonstrating the ability of skin DCs to elicit a cytotoxic anti-melanoma response will facilitate the development of novel anti-melanoma immunotherapies through direct targeting of cross-priming DC subsets.

The **aim** of this study was to test the ability of human skin APCs to cross-prime naïve CD8\(^+\) T cells against the melanoma-associated antigen, Melan-A, *in vitro*. 
Figure 1. Diagram of cross-priming. Migratory skin DCs uptake Melan-A antigen in the periphery and traffic to the draining lymph node. With the help of CD4+ T cells, DCs are licensed to present Melan-A antigenic peptide via their major histocompatibility complex (MHC) class I molecule to naïve CD8+ T cells. The naïve CD8+ T cells are cross-primed against Melan-A and enter the circulation as Melan-A-specific effector cytotoxic T lymphocytes (CTLs).
Methods

To test the ability of human skin APCs to cross-prime naïve CD8$^+$ T cells against the melanoma-associated antigen, Melan-A, I co-cultured naïve CD8$^+$ T cells from HLA-A*02$^+$ peripheral blood stem cells with allogeneic HLA-A*02$^+$ skin APCs loaded with 25µg/ml Melan-A protein overnight, for 9 days. For a positive control, skin APCs were loaded with Melan-A short peptide. For a negative control, skin APCs were not loaded with Melan-A protein or peptide.

Skin APCs and naïve CD8$^+$ T cells were isolated from plastic surgery patients’ surplus, healthy skin samples and peripheral blood stem cells respectively, by fluorescence activated cell sorting (FACS), see Figure 2.

After 9 days, the induction of Melan-A-specific cytotoxic T lymphocytes was determined by flow cytometry analysis of tetramer binding, see Figure 3.
Figure 2. Diagram of fluorescence activated cell sorting (FACS).
Figure 3. Diagram showing A) cross-priming of a naive CD8\(^+\) T cells against Melan-A by an antigen presenting cell (APC), B) the structure of an MHC class I/Melan-A peptide tetramer, and C) tetramer binding to a Melan-A-specific cytotoxic T lymphocyte (CTL).
Results and Discussion

I investigated the ability of five different skin APCs (dermal CD14<sup>+</sup> cells, CD1<sup>c+</sup> DCs, CD14<sup>1hi</sup> DCs, macrophages and epidermal Langerhans cells) to cross-prime naïve CD8<sup>+</sup> T cells against Melan-A in vitro.

The results from this study show that epidermal Langerhans cells (LCs) are capable of cross-priming naïve CD8<sup>+</sup> T cells against the melanoma-associated antigen, Melan-A, in vitro see Figure 4. Although it is difficult to prove unequivocally LCs are a cross-priming DC subset, these findings complement evidence showing LCs to be cross-presenting DCs<sup>14-16</sup>. There is ample evidence to support the hypothesis that LCs are efficient at cross-priming naïve CD8<sup>+</sup> T cells<sup>16-19</sup>.

The cross-priming ability of dermal CD14<sup>+</sup> cells, CD1<sup>c+</sup> DCs, CD14<sup>1hi</sup> DCs and macrophages in vitro was unconvincing due to the relatively high detection of tetramer<sup>+</sup> cells in the negative control, see Figure 4. However, without further investigation, the potential cross-priming ability of these dermal APCs cannot be disregarded.
Figure 4. Percentage of CD3$^+$CD8$^+$ T cells induced to tetramer$^+$/Melan-A-specific cytotoxic T lymphocytes after a 9 day co-culture of naive CD8$^+$ T cells with unloaded, Melan-A short peptide loaded or Melan-A protein loaded skin APCs. Composite data for two experiments is shown with the mean.
Conclusions

Dendritic cell (DC) mediated cross-priming of endogenous cancer-associated antigens is essential for the induction of *de novo* cancer antigen-specific cytotoxic T lymphocyte responses. DC subsets with functional specialisations have been identified in human skin, including migratory cross-presenting DCs but cross-priming DCs have not been isolated, despite their potential importance in immunity and generating an anti-tumour cytotoxic response.

This study shows epidermal LCs are capable of effectively cross-priming naïve CD8+ T cells against Melan-A *in vitro*. However, the number of donors is not adequate to reach statistical significance and merit LCs with the definitive status of a cross-priming DC subset; further investigation is required in order to consolidate these results. Ultimately, these findings will facilitate the development of novel anti-melanoma immunotherapies, through direct targeting of cross-priming DC subsets.
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References