Author's response to reviews

Title: A full scale comparative study of methods for generation of functional Dendritic cells for use as cancer vaccines

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Version: 3 Date: 15 May 2007

Author's response to reviews: see over
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Title: A full scale comparative study of methods for generation of functional Dendritic cells for use as cancer vaccines

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Version: 2  Date: 9 May 2007

Author's response to reviews: see over
Reviewer's report
A full scale comparative study of methods for generation of functional DCs for use Title:
as cancer vaccines

Version: 1 Date: 16 March 2007
Reviewer: Marc Dauer
Reviewer's report:

General
Based on a novel protocol for generation of monocyte-derived dendritic cells (DCs) in 48
hours established by Dauer et al. (FastDC; J Immunol. 2003 170(8):4069), the authors
have performed a comparative analysis of the FastDC protocol with a standard 7-day
protocol aimed at adaption for large-scale clinical use. They used elutriation to isolate
monocytes from leukapheresis products and a closed culture system with sterile
Teflon bags for DC generation. For antigen loading, the different DC preparations were
transfected with whole tumor cell RNA by electroporation. Transfection efficacy,
phenotype and T cell stimulatory capacity of FastDC and standard monocyte-derived DC
were compared. The authors claim that the 48-hour FastDC protocol yields DC with
equal quality and efficacy compared to a standard 7-day protocol and will be
implemented in clinical trials. The experiments described are generally well designed and
the results of considerable interest to the scientific community. The availability of a rapid
and reliable protocol for large-scale generation of DCs may facilitate evaluation of DC-
based tumor vaccination in clinical trials.

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Major Compulsory Revisions (that the author must respond to before a decision on
publication can be reached)

1) No statistical analysis has been performed for any of the experiments described.
Although the value of statistical analysis for the kind of experiments described is
debatable, the authors should at least provide information on how many independent
experiments were performed for each of the results described (figures 2 and 3).

We performed one full scale comparison for each of the 3 patients. Phenotyping by
flow cytometry was done once for each sample (immature and mature Fast- and
Standard DC (Fig 2) and EGFP transfection was done once for each donor (Fig 3).
One representative experiment was chosen for illustrative purposes in each of the
figures. Statistical analysis was not performed. The DCs from the patients were
used for clinical purposes, resulting in limited material for repeated experiments in
vitro. Information on the number of experiments is now given in the figure
legends.

2) In the first paragraph of the results section the authors described phenotypic
characteristics of FastDC that have already been reported by Dauer et al. (J Immunol.

A statement of this has now been included in the results section and a brief
discussion included in the discussion section with the proper reference. This
should be clearly stated and the respective citation referred to.

3) In the second paragraph of the results section, the authors state that FastDC retained
a monocytic phenotype (expression of CD14, low expression of CD1a and CD209) even
after 48 hours of culture with GM-CSF and IL-4. Dauer et al. have initially reported that
monocytes develop an immature DC phenotype with complete downregulation of CD14
already after 24 hours of culture with GM-CSF and IL-4 (J Immunol. 2003 170(8):4069). This finding has been confirmed in subsequent studies by the same group (Dauer et al., J Immunol Methods 2005 302(1-2):145-55). These controversial findings should be discussed briefly.

A brief discussion along the lines suggested by the reviewer (below), regarding the discrepancies by our findings and those of Dauer et al., has now been included in the discussion section.

One possible explanation could be the comparably high concentration of GM-CSF used by the authors that may promote development of a monocytic/macrophage-like phenotype in their DC cultures. Alternatively, the different processing of monocytes due to the use of elutriation technique and Teflon culture bags may lead to enhanced early activation of the monocyte precursors thus preventing a more rapid DC differentiation.

Minor Essential Revisions (such as missing labels on figures, or the wrong use of a term, which the author can be trusted to correct)

1) In the third paragraph of the results section, the authors present data from a so-called “wash-out” analysis, i.e. culture of DCs in the absence of cytokines or growth factors. They are able to show that FastDC retain their mature phenotype in the wash-out culture. This confirms previous findings by Dauer et al. described earlier (Dauer et al., J Leukoc Biol 2006 80(2):278-86). This should be briefly discussed and the respective citation included into the manuscript and referred to.

A brief discussion on the stability of the phenotype of Fast DC with reference to the work of Dauer et al., has now been included in the discussion, and the citation is included in the reference list.

2) To avoid misunderstandings, the second paragraph of the results section should be entitled “Immunophenotype of FastDC and Standard DCs”.

This paragraph has now been changed as suggested.

3) The authors do not explain how the results on viability of DCs described in figure 2 were obtained. This should be clarified.

It is now pointed out in materials and methods, under Wash out test.

Moreover, the graphic presentation of data in figure 2 should be improved. In the present form, data are not clearly visible and thus hard to interpret.

The graphics in Fig. 2 has now been improved.

4) Throughout the manuscript, the different DC preparations (FastDC vs. standard monocyte-derived DCs) are termed differently, either Fast DC or Fast DCs as wells Standard DC or Standard DCs. Terminology should be standardized.

This has been done, as suggested.
Furthermore, the abbreviation “DCs” should be avoided in the title of the manuscript.

It has been changed.

5) The manuscript deserves revision regarding correct use of the English language. There are several grammatical and spelling errors throughout the manuscript.

The manuscript has been revised regarding the English language.

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Discretionary Revisions (which the author can choose to ignore)
The authors do not provide any data on the quality of the monocyte preparation that is obtained by the elutriation technique as compared to other techniques such as plastic adherence or MACS technology (e.g. purity, viability). The quality of monocyte isolation may influence the quality of the DC preparation obtained after culture with GM-CSF and IL-4.

Data on monocyte yield, purity and viability referring to the elutration technique, has now been included in materials and methods section.

What next?: Unable to decide on acceptance or rejection until the authors have responded to the major compulsory revisions.

Level of interest: An article whose findings are important to those with closely related research interests.

Quality of written English: Needs some language corrections before being published.

Statistical review: No, the manuscript does not need to be seen by a statistician.

Declaration of competing interests:
I declare that I have no competing interests.

Reviewer's report
A full scale comparative study of methods for generation of functional DCs for use Title: as cancer vaccines
Version: 1 Date: 29 March 2007
Reviewer: Ingo Schmidt-Wolf

Reviewer's report:
General This is an interesting report on large-scale adaptation of the Elutra Cell Selection System for generation of DCs.

Major Compulsory Revisions (that the author must respond to before a decision on publication can be reached)

1. What is the efficiency of Jurkat E6 electroporation?
In the experiments using the whole mRNA fraction isolated from the Jurkat E6 cell line, we did not include a marker that allowed us to estimate the efficacy of transfection. What we did was to document the efficacy of transfection in parallel samples using EGFP mRNA. This allowed us to directly estimate transfection efficacy using the same transfection parameters and the same equipment by flowcytometry. In these parallel experiments, transfection efficacy, estimated as % transfected cells is >95%.

Statistics should be added to the methods and the result sections.

(See response to reviewer 1).

2. Figures 1 and 2: How many experiments were done?

Information on this has now been included in the Figure legends.

3. Figure 4: Data should be comprised as mean +/- standard error.

Mean and SD has now been included in Figure 4.

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Reviewer's report
A full scale comparative study of methods for generation of functional DCs for use Title: as cancer vaccines
Version: 1 Date: 2 April 2007
Reviewer: Anders Pedersen
Reviewer's report:

General In the manuscript by S J-Jankovic et al a full scale comparative study of two previously established methods for DC generation for cancer vaccination is performed.
DC based cancer vaccination is a promising strategy for the treatment of established cancer. A new fast method is compared with a method that has become standard in many laboratories. Since this fast method would make DC based cancer vaccination more rapid, less expensive and easier to perform it is important to evaluate this method in large scale production.

Major Compulsory Revisions (that the author must respond to before a decision on publication can be reached)

1. page 1, line 21: Not all the DC markers mentioned are DC marker, they are found on other cells as well.

This point has now been clarified in the text.

2. page 2, line 8: , followed by maturation... this sentence is grammatically problematic.

The sentence has now been changed, (see text).

3. page 2, line 13, In vitro.... It should be clarified that it is CD34+ cells from bone marrow etc that is used. Also, peripheral mononuclear cells are not a source, but monocytes from PBMC are!

This point has now been clarified in the text.

4. page 4, line 8: Why didn't the authors use the same time lenght for maturation

The aim of the preset experiments was to compare two different protocols. Difference in maturation time was one of the parameters that differ between the two protocols. In Fast DC (Dauer et al.), maturation time is 24 hrs, and in the standard protocol it is 48 hrs.

5. page 6, line 13: ELISPOT assay must be described in more details. Against which cytokine, against which cells. Was pre-stimulated cells used, or was a direct ELISPOT assay, etc ??

More details have now been included, as well as a reference (Jarnjak-Jankovic et al.) describing the same method in more detail.

6. page 6, line 18: Doesn't the length of cytoplasmic protrusions give some information on DC maturity and differences. This should be discussed.

Information on this point has now been included in the results section, and the discussion on this point has been extended in the discussion section.

7. page 8, line 19: significant should only be used if it its really statistically significant, based on significance tests!

This has now been changed.

8. The significance of different CD1a expression should be discussed.
It has now been included in the discussion.

Minor Essential Revisions (such as missing labels on figures, or the wrong use of a term, which the author can be trusted to correct)

1: page 1, line 24: The T cell response is allospecific and therefore polyclonal. I wouldn't consider this response a "specific T-cell" a term which I think should be used to describe T cell responses on a clonal level.

It is now changed.

2: page 4, line 11: were instead of was. Manus should be carefully checked for similar spelling mistakes.

This has now been checked through the whole manus.

3: page 5, line 8: Describe source of EGFP

It is now refered to (Saeboe-Larsen et al.)

4: page 5, line 16: Did the authors use Fc block, or why not?

We used Fc block, this is the standard procedure in our lab.

5: bonemarrow or bone marrow. Be consistent

It has been changed through the manus.

Discretionary Revisions (which the author can choose to ignore)

What next?: Unable to decide on acceptance or rejection until the authors have responded to the major compulsory revisions

Level of interest: An article whose findings are important to those with closely related research interests

Quality of written English: Acceptable

Statistical review: No, the manuscript does not need to be seen by a statistician.

Declaration of competing interests: I declare that I have no competing interests