The availability of a functional tumor targeting T-cell repertoire determines the anti-tumor efficiency of combination therapy with anti-CTLA-4 and anti-4-1BB antibodies

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Published in:
P L o S One

DOI:
10.1371/journal.pone.0066081

Publication date:
2013

Document version
Publisher's PDF, also known as Version of record

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Citation for published version (APA):
The Availability of a Functional Tumor Targeting T-Cell Repertoire Determines the Anti-Tumor Efficiency of Combination Therapy with Anti-CTLA-4 and Anti-4-1BB Antibodies

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Abstract

It has previously been found that combination therapy with anti-CTLA-4 and anti-4-1BB antibodies may enhance tumor immunity. However, this treatment is not efficient against all tumors, and it has been suggested that variations in tumor control may reflect differences in the immunogenicity of different tumors. In the present report, we have formally tested this hypothesis. Comparing the efficiency of combination antibody therapy against two antigenically distinct variants of the B16.F10 melanoma cell line, we observed that antibody therapy delayed the growth of a variant expressing an exogenous antigen (\(P < 0.0001\)), while this treatment failed to protect against the non-transfected parental line (\(P = 0.1850\)) consistent with published observations. As both cell lines are poorly immunogenic in wild type mice, these observations suggested that the magnitude of the tumor targeting T-cell repertoire plays a major role in deciding the efficiency of this antibody treatment. To directly test this assumption, we made use of mice expressing the exogenous antigen as a self-antigen and therefore carrying a severely purged T-cell repertoire directed against the major tumor antigen. Notably, combination therapy completely failed to inhibit tumor growth in the latter mice (\(P = 0.8584\)). These results underscore the importance of a functionally intact T-cell population as a precondition for the efficiency of treatment with immunomodulatory antibodies. Clinically, the implication is that this type of antibody therapy should be attempted as an early form of tumor-specific immunotherapy before extensive exhaustion of the tumor-specific T-cell repertoire has occurred.

Citation: Jensen BAH, Pedersen SR, Christensen JP, Thomsen AR (2013) The Availability of a Functional Tumor Targeting T-Cell Repertoire Determines the Anti-Tumor Efficiency of Combination Therapy with Anti-CTLA-4 and Anti-4-1BB Antibodies. PLoS ONE 8(6): e66081. doi:10.1371/journal.pone.0066081

Editor: Michael P. Bachmann, Carl-Gustav Carus Technical University-Dresden, Germany

Received March 13, 2013; Accepted May 1, 2013; Published June 13, 2013

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Funding: The project was funded by the Novo Nordisk Foundation. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors declare that they do not have any conflict of interest.

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Introduction

Following the overwhelming success of immunomodulatory antibodies in the treatment of autoimmune diseases, it is now time to fully exploit the potential of this class of potent drugs in the treatment of cancer. Several antibodies are already in clinical use, while others are under investigation in pre-clinical studies [1–3]. In this regard, antibodies against co-stimulatory molecules, such as cytotoxic T lymphocyte antigen 4 (CTLA-4) and 4-1BB, have emerged as potentially important therapeutics against various tumors [4,5].

CTLA-4 is a co-inhibitory receptor expressed on T-cells shortly after their activation [6], and it has been found to play an important role in the modulation of antigen-specific immune responses. In addition, expression of CTLA-4 is critical to the functionality of regulatory T-cells (Tregs) in vivo [7]. Collectively, blocking of this molecule allows for efficient stimulation of immune responses towards weak antigens, such as tumor antigens; however, it also increases the risk of self-reactivity, and studies in murine models have underscored this risk. Perhaps more importantly, autoimmune manifestations have also been observed in human patients [8], and careful clinical management is essential, if immune-related toxicities are to be kept acceptable [9].

4-1BB is a molecule belonging to the tumor necrosis superfamily. It is transiently up-regulated on T-cells subsequent to activation, and ligand binding is known to augment CD8 T-cell activity [10,11]. In various tumor models, agonistic anti-4-1BB antibodies have been found to improve tumor control [10]. Interestingly, even though 4-1BB signaling may render effector T-cells resistant to the inhibitory effect of Tregs [12], treatment with anti-4-1BB antibodies has also been found to reduce autoimmunity in lupus-prone mice [13].

Since agonistic anti-4-1BB antibodies appear to both improve anti-tumor responses and, in some cases, reduce autoimmunity, it has been suggested to combine this treatment with antibodies blocking CTLA-4 [4,5]. In fact, a study published by Kocak et al. seems to provide proof-of-concept in this respect [5]. Thus, these authors examined the efficacy of this combinatorial regimen in two distinct tumor models; MC38 colon carcinomas and B16 melanomas. Interestingly, they found that only MC38 challenged mice were significantly protected. As a plausible explanation for this, it was suggested that the difference in clinical effect might
result from differences in the intrinsic immunogenicity of the tested tumor cell lines. Yet, as only two very different tumor cell lines were studied, this explanation together with its implications could not be scientifically verified. Considering the clinical importance of...
developing new combinational treatments of human cancers, we decided to revisit the above subject and formally test whether the anti-tumor potential of combining these antibodies is in fact limited by the intrinsic immunogenicity of the involved tumor cells or whether it is more the availability of a functionally intact, tumor-specific T-cell repertoire, which is critical.

Accordingly, we made use of two closely related cell lines: wild type (WT) B16.F10 cells and a gene modified variant, B16.F10-GP, expressing the immunodominant epitope of the glycoprotein (GP) of lymphocytic choriomeningitis virus (LCMV) [14,15]. WT B16.F10 cells are poorly immunogenic, in part, due to weak MHC class I expression and a nonexistent MHC class II expression [16]. In contrast, B16 variants expressing exogenous transgenes are quite antigenic despite poor intrinsic immunogenicity, and for this reason they are commonly used to monitor the efficiency of, otherwise, e.g. vaccine, induced anti-tumor immune responses [4,14,17].

As an additional tool, we employed a mouse strain (Alb-1) expressing LCMV-GP as a self-antigen under the albumin promoter [18]. In these mice, the GP-specific CD8 T-cell repertoire is severely depleted, and, as a consequence, GP-specific responses in Alb-1 mice are greatly reduced compared to those induced in their WT counterparts [18].

Using the described experimental approach, we find that differences in the protective capacity of combinatorial therapy with antagonistic anti-CTLA-4 and agonistic anti-4-1BB antibodies do not so much reflect differences in the intrinsic immunogenicity of the tumor cells as the availability of a functionally intact T-cell repertoire targeting antigens expressed by the tumor cells.

Materials and Methods

Ethics Statement

Experiments were conducted in accordance with national Danish guidelines (Amendment #1306 of November 23, 2007) regarding animal experiments as approved by the Danish Animal Inspectorate, Ministry of Justice, permission #2011/561–87.

Mice

Alb-1-GP transgenic C57BL/6 mice were the progeny of breeding pairs originating from the animal facility of Spital, Zürich [18], and kindly provided by Daniel Pinschewer. WT C57BL/6 mice were purchased from Taconic M&B (Ry, Denmark).

Tumor Cell Lines

B16.F10 and B16.F10-GP (expressing the minimal epitope of the LCMV glycoprotein, GP33-41) melanoma cells were cultured...
relevant, vaccinations with human Ad 5-based vectors were
injected prior to inoculation. When the tumors reached the size of
6 mm diameter in the right flank at day 0. The mice were shaved at the injection
site and tumor volumes were calculated as length x width x 0.5236. When
relevant, vaccinations with human Ad 5-based vectors were
administered in the right hind footpad 5 days post tumor
inoculation.

Statistical Analysis
Comparison among groups in the survival experiments was
analyzed by the log-rank test (Mantel-Cox). Tumor volume are
presented as mean ± s.d. and analyzed by 2way ANOVA. Prism
6, GraphPad software (GraphPad Software Inc.) was used for all
statistical analysis. \( P < 0.05 \) was considered statistically significant.

Results and Discussion
Treatment with Agonistic Anti-4-1BB and Blocking Anti-
CTLA-4 Antibodies Augments Tumor Control
We have previously shown that the growth of B16.F10-GP
tumors can be partially controlled by therapeutic vaccine with
Ad5 vectors expressing GP [17]. We could also demonstrate that
tumor control was markedly improved if GP was tethered to the
MHC class II associated invariant chain (Ii), designated Ad5-IiGP, was produced as
described previously [20].

Injections and Tumor Measurements
All mice were subcutaneously injected with \( 10^6 \) melanoma cells
in the right flank at day 0. The mice were shaved at the injection
site prior to inoculation. When the tumors reached the size of
\( \geq 12 \) mm, the mice were euthanized for ethical reasons. The
tumor volumes were calculated as length x width x 0.5236. When
relevant, vaccinations with human Ad 5-based vectors were
administered in the right hind footpad 5 days post tumor
inoculation.

Results and Discussion
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described previously [20].

Gene Modified B16.F10 Cells are much more Susceptible
to Antibody-induced Tumor Control than their WT
Counterparts
As our results seemingly conflicted with those of Kocak et al.
[5], describing that this antibody combination was insufficient for
treatment B16 tumors, we hypothesized that the success of this
antibody treatment relates to the presence of a “strong” foreign
antigen on the tumor cells combined with a substantial population
of matching T-cells. This would not be the case for WT tumor
cells, which would not be expected to express any strong antigens.

To test this prediction, we injected WT mice with \( 10^6 \) B16.F10
cells, with or without expression of exogenous antigen (GP),
followed by antibody treatment. The results presented in
Figure 1B–C clearly support our hypothesis: antibody treatment
did not significantly impact tumor growth in B16.F10 challenged
mice (\( P = 0.1850 \)); whereas the effect on B16.F10-GP challenged
mice were highly significant (\( P < 0.0001 \)). Untreated mice were
equally susceptible to both cell lines (\( P = 1.000 \)).

The Antibody-induced Control of B16.F10-GP Cells is
Abolished in Mice Carrying GP as a Self-antigen
The observed difference in the efficiency of antibody therapy
against tumor cells bearing exogenous antigen versus no antigen
underscored a role for functionally intact, tumor-specific T-cells in
the antibody induced tumor control. Although overall T-cell
depletion of course would validate a role of T-cells, this treatment
would not provide any information regarding the fine specificity
requirements of the involved cells. For this reason, we decided to
use a subtler approach to test whether successful antibody induced
tumor control requires a functionally intact population of tumor-
specific CD8 T-cells. Thus, we used Alb-1 mice, which express GP
as a self-antigen and for that reason have a severely depleted T-cell
repertoire for this antigen compared to WT mice [18].

As predicted, if an intact tumor targeting T-cell population play
a decisive role when it comes to the efficiency of this antibody
combination, Alb-1 mice, unlike WT mice, were equally
susceptible to tumor challenge with B16.F10-GP cells whether they
received antibody therapy or not (\( P = 0.8584 – Figure 2 \)).

Concluding Remarks
The results of the present study confirm that this antibody
combination is inefficient against the WT B16 cell line. In contrast,
the antibodies could efficiently delay the growth of a variant of this
tumor cell line expressing a foreign antigen. Since the two cell lines are
identical except for the presence of the transgene, and there is
clear evidence in the literature that neither of these cell lines are
very immunogenic when inoculated into normal WT mice [15],
our results strongly indicate, that availability of tumor-targeting T-
cell repertoire represents a key factor in deciding the clinical
efficiency of combination therapy with anti-4-1BB and anti-
CTLA-4. Furthermore, the efficiency of antibody treatment
clearly relates to the size of the T-cell repertoire targeting antigens
expressed by the tumor cells. Thus, in mice (Alb-1) with a T-cell
repertoire purged of most T-cells specific for the major tumor
antigen relevant under the current test conditions (GP) [18],
combined antibody treatment did not significantly delay the
growth of GP-expressing tumor cells. Some might argue that it is
self evident that the presence of tumor-targeting T-cells represents

PLOS ONE | www.plosone.org 4 June 2013 | Volume 8 | Issue 6 | e66081
a precondition for immunomodulatory antibodies like anti-CTLA-4 and anti-4-1-BB to have an effect on tumor growth. However, this insight is often ignored or forgotten in actual clinical practice. Thus, since cancer immunoediting is assumed to rapidly remove the most immunogenic cancer cells [22], the present findings serve to underscore that this antibody combination therapy is likely to be efficient only in cases where the remaining low-immunogenic tumor cells express tumor antigens for which there is little or no purging of the naive T-cell repertoire, i.e, neoantigens representing mutated self or viral antigen. Furthermore, as prolonged cancer growth is believed to be associated with functional impairment of relevant T-cells [23,24], results like ours tend to imply that immunomodulatory antibody treatment should be tested as an early treatment modality – before T-cell exhaustion is severely progressed - and not as a last resort.

Author Contributions
Conceived and designed the experiments: BAHJ JPC ART. Performed the experiments: BAHJ SRP. Analyzed the data: BAHJ JPC ART. Wrote the paper: BAHJ ART. Revised the paper: SRP JPC.

References