Reviewer's report

Title: Evaluation of Immunological Escape Mechanisms in a Mouse Model of Colorectal Liver Metastases

Version: 1 Date: 6 August 2009

Reviewer: Sara Michel

Reviewer's report:

In the manuscript entitled „Evaluation of immunological escape mechanisms in a mouse model of colorectal liver disease“ by Grimm et al., the authors study mechanisms of immune evasion in a mouse model of colorectal liver metastasis at different time points of tumor growth. Especially in the light of recent findings on the significance of immune responses for the course of the disease in colorectal cancer, tumoral immune evasion is a relevant subject.

Major compulsory revisions:

1. p 8 l24: Normal liver tissues used as “naïve controls” need to be described in greater detail: were these animals comparable to those used in the experiment (age/weight/sex/strain - any pretreatments?)

2. p8: A description of how the percentage of immunohistochemically stained cells was determined is lacking and needs to be added to the method section. Likewise, the evaluation of immune-fluorescences should be described.

3. p11: RT PCR: How was amplification of genomic DNA excluded?

4. p13 l19: It is stated that seven animals per group were used for RT experiments. However, in the statistics section of the methods, it is described that the analysis was performed on five animals per group. This needs to be clarified. Also, it does not become clear why animals at day five after injection were not included in the analysis. These data should be presented as well. See also p12 l20.

5. Figures 2 - 4: SD and ranges should be provided in the Table below each Figure. For all Figures, it has to be indicated (e.g. by a line) which comparisons exactly were found to be significant. Values for day 5 post-OP need to be presented.

6. Figures 2C, 4D: The proportion of immunoreactive cells appears to be very high. Moreover, the single staining for CD8 (Figure 4D, middle upper panel) seems to have a nuclear component. This might be attributed to thickness of the specimen or non-specific background staining. The authors should provide conventional IHC single stainings for CD8 and CD4 in order to give a better impression of T cell infiltration. This would also help to judge on histology of the stained tissue. Unfortunately, the dotted lines and asterisks provided are not helping to that end (asterisks being truncated, too).
7. Figure 2B: From the table provided it looks like about 59% of cells were T cells (34% CD4+ and 25% CD8+) in the tumor at day 20 after tumor cell injection. This number appears very high. IHC stainings need to be provided (see also comment to Figures 2C, 4D) and this finding needs to be discussed.

8. Discussion: A large proportion of the discussion is speculative. The authors need to be careful about the lines of argumentation used and should keep in mind that their results are (i) observatory and (ii) derived from an artificial animal model. Parts of the discussion should be rewritten and toned down.

Examples:

p16 l13-16: The sentence needs to be rewritten, because it implies an active mode of suppression exerted by (defective) cytotoxic T cells.

p16 l19-22: It is unclear what the sentence refers to (“This indicates”). The results presented in the present manuscript are strictly descriptive. The mere upregulation of TNF-a does not allow drawing conclusions on its mode of action. Moreover, with the methods applied, TNF-a production can not be attributed to the tumor cells, but might as well be produced by infiltrating immune cells. The sentence needs to be toned down and references for the assumed mechanisms should be provided.

p17 l13-15: The authors observed higher numbers of T cells in more advanced metastases. Although the possibility exists, this does by no means demonstrate that theses cells were attracted by cytokines or other factors.

9. p15 l23: It is unclear what is meant with “biologically accurate” and “biological sufficient”. This expression should either be specified or omitted.

The model used does not allow to analyze initial steps of metastasis formation (highly likely to result in variants altered due to immunoselection) or to analyze intrahepatic lesions after longer periods of time due to rapid tumor growth. These limitations should be discussed.

Minor essential revisions:

1. Basic and well established mechanisms of immune evasion like for example alterations in the cellular antigen processing and presentation machinery (loss or downregulation of MHC I, downregulation of APM components) have not been studied. The authors might want to comment on classical mechanisms of immune evasion in introduction and discussion.

2. Abstract p2 l17, l18: The expression “stage-dependent” is misleading and should be replaced.

3. p10 l10: The authors state that they analyzed six HPF representative of the whole tumor section. However, on p8 l6 it reads like biopsies were only taken from the margin of the metastasis. Given the known heterogeneity of lymphocyte infiltrates between margin and center of metastasis, the authors might want to comment on that.
4. p10 l11-12: The sentence needs to be rewritten.

5. p13 l5-9: The description of injection with 1x10^6 tumor cells does not add to the results.

6. p13 l26 “increasing” should be replaced, because biopsies from different animals were used at different points of time.

7. Figure 1: SD of tumor volume for time 5 days is lacking. The statement “in all animals” should be omitted.

8. Figure 2a, 4a: “Metastatic liver” should be changed to “metastatic tissue”.

**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'