Reviewer's report

Title: Distinct mechanisms of loss of IFN-gamma mediated HLA class I inducibility in two melanoma cell lines.

Version: 1 Date: 25 January 2007

Reviewer: Catia Traversari

Reviewer's report:

- Major Compulsory Revisions
  1. The DIFFERENCES in the molecular mechanism used by IFN-gamma with respect to IFN-alpha to control MHC-I expression must be described in more detail in the introduction.

  2. Fig 2 the phospho-STAT1 detected upon stimulation with IFN-gamma seems to have a lower MW in ESTDAB-159 cells than in Control cells: please comment on this matter.

  3. In the ESTDAB-159 cells the IRF-1-regulated genes EGFR and iNOS were not induced by IFN-gamma treatment. However, induction of EGFR lacks a positive control since it is already expressed at basal level by the control cells used in the experiment. I suggest to use a more suitable cell line as positive control or to remove the analysis for EGFR expression.

  4. Differently from HLA-I, increased levels of EGFR expression were detectable after the treatment with AZA alone, while the addition of IFN-gamma did not further increase the EGFR expression. The authors should discuss this matter.

  5. It is unclear which is the treatment that the authors refer to in the last sentence of the discussion “…prediction of the immune response in patient treated with long-term IFN”. Please add a reference.

- Minor Essential Revisions
  1. Pg 2 line 15, and pg 15 line 3 and 4: INF?IFN

  2. Pg 2 line 2: the sentence “Examination of STAT-1 in this cell line” is unclear. Please specify: “Examination of STAT-1 in ESTDAB-004”.

  3. Pg 3 Last line: IRF-1 should be changed in IFN-regulatory factor 1 (IRF-1)

  4. Pg 4 last line: IIFN-g ? IFN-g

  5. Pg 5 line 4: ISGF3 is the abbreviation for…..

  6. Pg 10: Is the reported absence of inducibility for MHC-II molecules (data not shown) referred to IFN-gamma, IFN-alpha or both?

  7. Figure 1A: in the plot the y axis lacks description. More details (eg GUBS) on the RT-PCR analysis reported in panel A, should be included in the legend.

  8. Pg 10: the sentence “…showed a similar STAT1 protein level to that detected in the control line (figure 2)” is misleading. Indeed, the STAT1 expression level of the Control cell line is reported ONLY in the panel IFN-alpha/ESTDAB-004 of the figure 2, this should be indicated in the text.

  9. Fig 4’s legend: …IRF-1 (A), iNOS, and EGFR (A). ? IRF-1, iNOS, and EGFR (A)

  10. Figure 6A: the y axis of the plot lacks description.

  11. Figure 6B: the lines (dotted, full etc) used to indicate the histograms of the negative control, the HLA-ABC and the HLA-ABC+ AZA, can not be distinguished in the figure.

  12. There is not consistency in the cell line reported as positive controls in the different experiments. In
figure 5 the name of the cell line used as positive control (ED56) is reported in the figure. In figure 4 the
name (ESTDAB-108) is reported only in the discussion. For the other figures no indications concerning the

cell line used as positive control are reported.

- Discretionary Revisions
1. It should be interesting to show the results of Jak2 phosphorilation in response to IFN-gamma and the
basal level of SOC1 in ESTDAB-159

2. Figure 4 showed that in ESTDAB-159 cells the IRF-1-regulated genes EGFR and iNOS were not induced
by IFN-gamma treatment. It would be nice to show in the same figure also the absence of induction of
HLA-I mRNA, even if the results have been already shown as plot in fig 1.

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