Addendum to:

Materials and methods

Cell culture

Murine Lewis lung carcinoma (LLC) cells, originally derived from a spontaneous tumor in a C57BL/6 wild-type mouse (22), were obtained from American Type Culture Collection (Manassas, VA). The LLC cells were cultured under standard conditions in high glucose DMEM (Gibco Invitrogen Cell Culture, Carlsbad, CA) with 10% fetal bovine serum (FBS) (Gibco Invitrogen Cell Culture) and 5% CO₂.

Tumor Injections

For tumor growth analysis C57BL/6 wild-type male mice (Jackson Laboratory, Bar Harbor, Maine), and Balb/C wild-type mice (Taconic) were injected s.c. in the mid dorsum with 10⁶ murine Lewis lung carcinoma cells. Tumor size was measured regularly with calipers, and animals were sacrificed when tumors reached 1.5 cm³.

Tissue processing

Mice were sacrificed with a 0.6 ml intraperitoneal injection of 2,2,2-tribromoethanol at 20 mg/ml. Tissues to be frozen-sectioned were dissected and slow-frozen in OCT (Tissue Tek, Fisher Scientific, Pittsburgh, PA) in the gas phase of liquid nitrogen. Tissues to be paraffin-sectioned were placed in 10% formalin, processed by standard protocol (23), placed in cassettes, and paraffin-embedded. Paraffin-embedded tissues were cut into 4 μm slices, placed on
positively charged slides (Fisher Scientific), and stained with hematoxylin and eosin (H&E) using standard protocols (23).

As seen in Figure 2, the dynamics of the LLC tumor progression varied dramatically between the syngeneic C57BL/6 mice and the Balb/C strain. Roughly exponential growth is seen in the C57BL/6 background, while the same murine carcinoma LLC falters in a complicated fashion before ultimately being rejected in the wild-type Balb/C mice due to immune response (Figure 2B). Intriguingly, while one might expect direct tumor elimination due to the elicited immune response to LLC in the Balb/C mice, a very significant initial spike in tumor growth was observed before tumors ultimately regressed. Included in our analysis is a reconciliation of these growth dynamics.

**Mathematical Model**

Full description of Model (1) can be found in Kareva and Berezovskaya (2015).

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\begin{align*}
\frac{dT_a}{dt} &= r_T a(T_a(t)) \frac{G(t)}{1+b_T G(t)} e_a \frac{T_a(t)I(t)}{I+s(T_a(t)+T_g(t))} \frac{T_a(t)}{n}\text{atural death} \\
\frac{dT_g}{dt} &= r_T g(T_g(t)) \frac{G(t)}{1+b_T G(t)} e_g \frac{T_g(t)I(t)}{I(t)+s(T_a(t)+T_g(t))} T_g(t) \\
\frac{dG}{dt} &= (G_0 + G(t)) \left( d_a T_a(t) + d_g T_g(t) \right) \frac{G(t)}{1+b_T G(t)} d_I(t) \frac{G(t)}{1+b_I G(t)} \\
\frac{dI}{dt} &= (i_0(T_a(t)+T_g(t))) \frac{I(t)}{1+b_I G(t)} \left[ I(t)(T_a(t)+T_g(t)) \right. \\
& \quad \left. + r_I I(t) \right] \frac{I(t)(T_a(t)+T_g(t))}{(I(t)+s(T_a(t)+T_g(t)))} \\
& \quad \text{cell expansion stimulated by debris from previously killed tumor cells and modulated by glucose}
\end{align*}
\]
Results

An important goal in this investigation is to replicate experimentally observed tumor growth dynamics using the mathematical model, proposed in (Kareva and Berezovskaya, 2015). The reported growth curves can indeed be replicated by our model, and the difference between tumor escape (Figure 3A) and tumor regression (Figure 3B) can be mechanistically explained either through insufficient immunogenicity, as represented through variations in parameters $i_0$ and $e_j$ (Figures 3C,D), or through elevated immune cell mortality as represented by parameter (Figure 3E,F). Interestingly, in the case of insufficient immunogenicity, the model predicts a characteristic spike (Figure 3D), which closely resembles the spike observed experimentally (Figure 3B). A closer inspection of the underlying dynamics through the mathematical model reveals that the spike appears due to the rise and subsequent rapid regression of the subpopulation of glycolytic cancer cells, which are gradually outcompeted by the aerobic cancer cells. Smoother growth curves, such as observed in Figure 3F, proved to be generated primarily by glycolytic cancer cells, which do not go through a rapid regression phase and which do not become dominated by the aerobic cancer cells over time.
Figure 1. Proposed scenario of metabolism-driven tumor escape. In the tumor microenvironment, glucose is taken up by cytotoxic lymphocytes that use glycolysis as a primary mode of metabolism, causing the tumor to contract and exposing its anaerobic core. The glycolytic cancer cells have up-regulated nutrients transporters, thus posing competition to immune cells for resources. Lymphocytes cannot proliferate unless their nutrient demands are met, which can allow tumor cells to circumvent immune response, leading to tumor escape.
Figure 2. Tumor progression in different immunocompetent mice. (a) H&E images of LLC tumors corresponding to tumor growth plot for C57BL/6 mice. (b) LLC cells originated from C57BL/6 background mice and naturally grow in C57BL/6 mice, while (c) the immune system of the wild-type Balb/C mice rejects the LLC cells. The x-axis represents the time after cells were injected. Experiments reported here were conducted by Afshin Beheshti.
Figure 3. Dynamics of LLC cell growth in (a) C57BL/6 mice and (b) Balb/C mice, compared to dynamics predicted by the mathematical model. Differences in (c) and (d) arise due to variations in parameters of immune stimulation $i_0$ and tumor elimination $e_j$, while differences in (e) and (f) arise due to variations in parameter of immune cell mortality $\mu_I$. 
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