

Innate predisposition to immune escape in follicular lymphoma cells

Pauline Gravelle,^{1,2,3} Christine Jean,^{1,2,3} Wilfried Valleron,^{1,2} Guy Laurent^{1,2,3,4} and Jean-Jacques Fournié^{1,2,3,*}

¹INSERM UMR1037; Cancer Research Center of Toulouse; Toulouse, France; ²Université Toulouse III Paul-Sabatier; Toulouse, France; ³ERL 5294 CNRS, BP3028, Hospital Purpan; Toulouse, France; ⁴Department of Haematology, Hospital Purpan; Toulouse, France

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Abbreviations: MALC, multicellular aggregates of lymphoma cells; IDO, Indoleamine 2,3-dioxygenase

Tumor-intrinsic immuno-resistance is a prerequisite for emergence of follicular lymphomas. Here we show that in vitro, such cells are more resistant to immune cytotoxicity when grown as follicle-mimicking tridimensional aggregates than when grown as cell suspensions. So in patients, this innate adaptation to tumor immunity might precede its selective pressure.

The cure of non-Hodgkin lymphomas (NHL) largely depends upon cytotoxic lymphocytes recruited during therapy, but most NHL cells appear relatively resistant to their action. It is thus important to understand how they do so. A hallmark of follicular lymphoma is their growth as dense aggregates in lymph nodes, a feature however lacking from all in vitro studies which currently involve cell suspensions only.

Here we grew lymphoma cells in vitro as tridimensional multicellular aggregates (MALC) to test whether such a mode of cell growth promotes some degree of immuno-resistance. In this aim, MALC of the t(14,18)⁺ follicular lymphoma cell line RL were produced in vitro by a modified “hanging drop” method.^{1,2} This type of cell growth formed compact ovoid aggregates reaching a diameter of ~1 mm by three weeks of culture, which were never produced in the usual cell suspensions in culture flasks. We then asked whether these two kinds of cell growth induced different gene expression profiles. So, the transcriptomes from RL cells grown for 10 d either as MALC or cell suspensions were produced on Affymetrix microarrays (HG-133 plus 2.0) and

analyzed by Autocompare.³ 451 genes were significantly upregulated in cells grown as MALC comparatively to cell suspensions ($> 4\times$, $p < 0.05$). These were enriched for genes upregulated in the central area of tumors relative to periphery ($p = 10^{-20}$)⁴ and for genes regulated by the histone-lysine N-methyltransferase gene EZH2 ($p = 10^{-12}$).⁵ The upregulated MALC genes also reflected the highly relevant hallmarks of follicular lymphoma cells: induction of anti-apoptotic functions ($p = 0.0002$), pro-survival NF κ B cascade ($p = 0.0006$) and response to hypoxia ($p = 0.002$). Further, the 241 most downregulated MALC genes ($< 0.5\times$, $p < 0.05$) indicated shut down of cytokine/cytokine receptor interactions ($p = 10^{-7}$) and chemokine signaling ($p = 10^{-5}$). So, lymphoma cells grown in vitro as MALC present a gene expression pattern which is not only highly relevant of follicular lymphoma cells in vivo, but also suggests a pre-adaptation to selective pressure from immunity. This view was strengthened when the lymphoma cells grown either in culture flasks or MALC were dissociated and compared as targets in classical 4 h ⁵¹Cr-release lysis assays by allogeneic NK cells from different

healthy donors. The lymphoma cells were more resistant to NK lysis when grown as MALC than when grown as cell suspensions (Fig. 1).

Various lymphoma cell-intrinsic immuno-escape pathways such as altered HLA-I, CD58,⁶ IDO⁷ and deficient immune synapses⁸ have been identified. We now propose that growth as cell aggregates represent another tumor cell-intrinsic pathway of immuno-resistance for follicular lymphomas. The transcriptome of follicular lymphoma cells grown as MALC was characteristic of deeply intra-tumoral cancer cells⁴ and reflected regulation by known drivers of lymphomagenesis.⁹ Together with these features, cells from MALC showed more anti-apoptotic and “immunosilent” gene expression profiles and they showed higher resistance to NK lysis than cells in suspension. So when grown aggregated in vitro, the lymphoma cells show a remarkable tumor-intrinsic pre-adaptation to further immune pressure. These observations suggest that natural lymphomagenesis in lymphoid organs from patients might also involve such an “innate” predisposition to immune escape.

*Correspondence to: Jean-Jacques Fournié; Email: jean-jacques.fournie@inserm.fr
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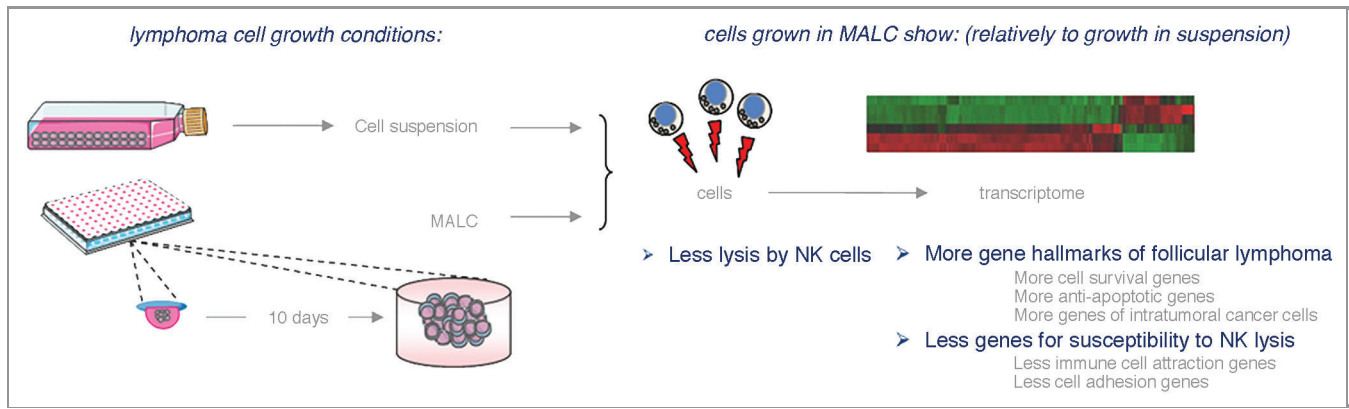


Figure 1. Follicular lymphoma cell growth in vitro as MALC increases immune resistance.

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