Charlebois and colleagues recently reported that the antitumor activity of anti-ErbB2 mAb is enhanced by local polyI:C and CpG administration in murine breast tumor models (1) and concluded that this activity was dependent on IFNs, CD8^+ T, and natural killer (NK) cells. The requirement for NK cells was based on in vivo depletion using anti-asialo-GM1 (ASGM1) Ab.

**Letter to the Editor**

TLR-3/9 Agonists Synergize with Anti-ErbB2 mAb—Letter

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ASGM1 is a glycolipid highly expressed on the surface of mouse NK cells, but also expressed on T cells, NKT cells, eosinophils, basophils, and macrophages (2). Treatment with anti-ASGM1 Ab reduces NK-cell cytolytic ability in vitro, and in vivo, results in NK depletion by an unexplained mechanism (3). Anti-ASGM1 is available as polyimmunoglobulin (pIg) or IgG preparations. Most published studies, including Charlebois and colleagues’ work, use the pIg. Our studies show that anti-ASGM1 pIg binds to macrophages at a higher level than anti-ASGM1 IgG (Fig. 1A and B) and that the IgG preparation is more efficient at depleting NK cells (Fig. 1C and D). When mice were treated with 50, 25, or 10 μL anti-ASGM1 on day 1, 10 μL of anti-ASGM1 IgG was as efficacious as 50 μL of pIg in depleting peripheral and splenic NK cells. Despite anti-ASGM1 binding to myeloid cells, we did not observe any significant changes in the number of these cells (Fig. 1E).

Next, we examined the effects of anti-ASGM1 on macrophage function. Anti-ASGM1 pIg, but not IgG (at 10 μg/mL or a supersaturating level of 100 μg/mL), impaired macrophage-mediated phagocytosis of anti-CD20 (4)—opsonized human CD20-transgenic B cells in vitro (Fig. 2A and B). This effect was absent with deglycosylated anti-ASGM1 pIG, indicating that FcR-dependent effector function was impaired. In vivo, anti-ASGM1 pIg reduced anti-CD20-mediated B-cell depletion to 60% in some mice (Fig. 2C). In contrast, the IgG preparation induced a less profound effect, especially at 10 μL, reducing depletion by only 10% at 48 hours.

In summary, we demonstrate that the anti-ASGM1 Ab used by Charlebois and colleagues can disrupt macrophage function. Although we do not dispute that NK cells might have a role in the described antitumor activity, our data suggest that the authors may have inadvertently excluded the contribution of macrophages, which can be affected by pIg anti-ASGM1 and which are known to be critical mediators of mAb function (5). Therefore, we highlight an important caveat of using the pIg preparation of anti-ASGM1 to deplete NK cells and recommend the use of a pure IgG preparation.

**Disclosure of Potential Conflicts of Interest**

No potential conflicts of interest were disclosed.

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