JEM Journal of Experimental Medicine

INSIGHTS

CD81 as target for B cell lymphomas

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In this issue of *JEM*, Vences-Catalán et al. (https://doi.org/10.1084/jem.20190186) demonstrate that a particular anti-CD81 antibody shows promising features as a novel immunotherapeutic tool to treat B cell lymphomas. Surprisingly, although CD81 is widely expressed, only minor side effects on other CD81⁺ immune cells analyzed were observed.

CD81 is a member of the tetraspanin family, which encompasses membrane proteins characterized by four transmembrane domains (Levy, 2014). CD81 is expressed by many cells of the hematopoietic system, including B cells, T cells, monocytes, and dendritic cells. The functions of CD81 are multifaceted, including roles in immune synapse formation, adhesion, migration, receptor clustering, and signaling. In B cells, CD81 forms a complex with CD19 and CD21 and thereby lowers the threshold for B cell receptor activation.

It was nearly 30 yr ago that, in a screen for antibodies able to kill lymphoma cells, Levy and colleagues raised a monoclonal antibody that later turned out to recognize CD81 (Oren et al., 1990). This clone, 5A6, a mouse anti-human IgG1 antibody, is the basis for the present work by Vences-Catalán et al. In the initial experiment of the study, injection of the 5A6 antibody reduced tumor burden in a murine xenograft model with human B cell lymphoma lines. 5A6 has a direct cytotoxic effect in the lymphoma cells, evidenced by activation of caspase-3. Importantly, this is not a general feature of anti-CD81 antibodies, because three other monoclonal anti-CD81 antibodies did not induce cell death. As direct cytotoxicity induced death in only a fraction of cells in vitro, it was studied whether 5A6 has further effector functions. Adding natural killer (NK) cells to the in vitro assay revealed that 5A6 also efficiently mediates antibody-dependent cell cytotoxicity (ADCC). Considering that mouse IgG1 is not efficient in activating complement, Vences-Catalán et al. (2019) tested whether the killing efficiency of 5A6 could be augmented by substituting the constant region of IgG1 heavy chain with either mouse IgG2a or human IgG1 constant regions. Indeed, both modified 5A6 antibodies showed strong complement-dependent cytotoxicity (CDC), whereas the original mouse IgG1 5A6 showed little CDC. The two variants of 5A6 were also more efficient than the original clone in mediating antibody-dependent cellular phagocytosis (ADCP) while retaining efficient ADCC effector functions. The improved efficiency of the two 5A6 variants in terms of target cell killing was not only detectable in vitro, but was also seen in the xenograft model with the Raji lymphoma cell line. Depletion of either macrophages, NK cells, or complement in the xenograft model revealed that macrophages and NK cells both substantially contributed to the in vivo activity of 5A6, indicating major roles of ADCP and ADCC. As expected from the in vitro experiments, complement had no effect on the activity of 5A6 in vivo. Overall, the 5A6 anti-CD81 antibody and its two variants show an impressive efficiency in killing human B cell lymphoma cell lines, involving direct cytotoxicity, ADCP, and ADCC, and for the variants, additionally CDC.

A major concern for the application of anti-CD81 antibodies to patients is the widespread expression of CD81 by cells of the immune system, but also cells in various tissues. It is therefore essential to study in vitro, but also in a preclinical mouse model, potential detrimental effects of this antibody on non-lymphoma cells. In a first analysis in this direction, several lymphoma cell lines showed higher expression of CD81 than peripheral blood mononuclear cells, and upon application of 5A6 to co-cultures of Raji cells with blood mononuclear cells or purified B cells, the nontumor cells were largely resistant to killing by CDC or ADCC, whereas the Raji cells were efficiently killed. Moreover, application of all three versions of 5A6 to a transgenic mouse line expressing human CD81 on various types of cells did not result in severe health problems. Finally, cell suspensions from six biopsies of follicular lymphomas were incubated in vitro with the murine IgGa2 5A6 antibody and human serum. In each of the biopsies, the follicular lymphoma cells were efficiently killed, whereas the nontumor T cells from the lymphoma microenvironment were minimally affected. The increased sensitivity of the lymphoma cells may partly be due to the observation that they show reduced expression of the complement inhibitor CD55, so that CDC might be stronger for the lymphoma cells

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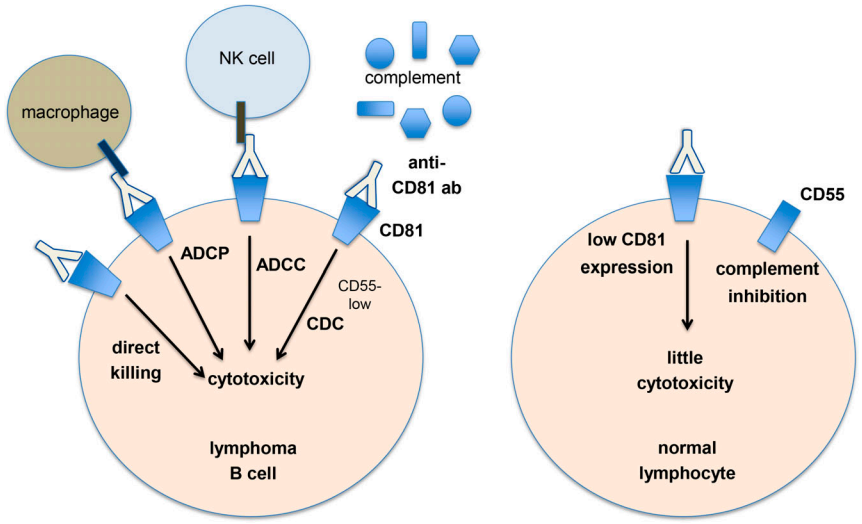

Binding of the anti-CD81 monoclonal antibody 5A6 to CD81 on lymphoma cells expressing high levels of this tetraspanin induces a strong cytotoxic effect, which is mediated by several mechanisms. Besides a direct apoptosis-inducing effect, 5A6 promotes ADCC through NK cells and ADCP through macrophages. The murine IgG2a and the human IgG1 5A6 variants also show strong CDC. Normal lymphocytes are much less sensitive to the anti-CD81 antibody (ab). This is likely partly due to a lower expression level of CD81 and higher expression of the complement inhibitor CD55 on normal lymphocytes in comparison with lymphoma B cells.

than for normal lymphocytes. Taking these safety studies together, anti-CD81 antibody treatment seems to be less toxic for nontumor cells than for lymphoma B cells.

The data presented in the comprehensive work by Vences-Catalán et al. (2019) are promising and might even be of relevance beyond the therapy of lymphomas, because CD81 is also highly expressed on many solid cancers, so that such tumors may principally be considered for an immunotherapy targeting CD81 (Vences-Catalán et al., 2017). Nevertheless, a number of caveats need to be considered, and further studies are essential before it will become clear whether anti-CD81 immunotherapy is indeed a suitable therapeutic strategy for B cell lymphomas. One caveat is that the present work was largely restricted to a few lymphoma cell lines expressing high levels of CD81, and primary lymphoma cells from only a few follicular lymphomas were studied in one experiment. It seems that CD81 is heterogeneously expressed by B cell lymphomas, both in terms of the fraction of cases positive and the CD81 expression levels on the lymphoma cells (Luo et al., 2010; Cardoso et al., 2018; Vences-Catalán et al., 2019). Thus, it will be important to determine which types of B cell lymphomas are amenable to therapy by anti-CD81 antibody and which expression levels of CD81 are essential to allow an efficient elimination of the lymphoma cells. Moreover, considering that lymphoma cells often do not express much higher levels of CD81 than normal lymphocytes, it is puzzling that the lymphoma cells appear to be much more sensitive to the anti-CD81 antibody. The reduced level of the complement inhibitor CD55 may partly account for this, as mentioned above, but considering that the anti-CD81 antibodies confer their cytotoxicity not only through CDC, but also through three further mechanisms, one wonders whether there are additional factors that account for the higher sensitivity of the lymphoma cells than normal lymphocytes.

For a target antigen that is not consistently expressed by tumor cells of a type of lymphoma and that does not seem to be essential for survival of B cells (van Zelm et al., 2010), there might be a particular high risk of development of resistance against the targeted therapy, e.g., by down-regulation of CD81 in a fraction of lymphoma cells, which are then selected to survive. A further concern is that it is still unclear whether an anti-CD81 antibody therapy of human patients may cause severe side effects, although the initial safety studies in the work by S. Levy and her team are promising (Vences-Catalán et al., 2019). As CD81 is expressed by many types of immune cells, complex consequences of a long-term application of the antibody may be expected. For example, among normal lymphocytes, CD81 shows highest expression in germinal center B cells (Luo et al., 2010), so that these cells may be eliminated, resulting in compromised humoral immune responses during therapy with anti-CD81 antibodies. Moreover, CD81 plays a role in the immunosuppressive function of regulatory T cells and myeloid-derived suppressor cells in regulating tumor growth and metastasis (Vences-Catalán et al., 2015). Depending on whether anti-CD81 antibodies have stimulatory or cytotoxic effects on these cells, these potential side effects may either support or weaken the therapeutic effect of the treatment.

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