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Editorial

Editorial: **Natural killer cell-mediated augmentation of autoantibody production?**

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In past decades, NK cells, originally assigned only to the innate immune system, have been recognized to influence various aspects of adaptive immune responses. As such, activated NK cells are capable of initiating constant region switch recombination in B cells by inducing an IgG 2a germ line as well as activation-induced cytidine deaminase transcripts in vitro [1] . This effect was shown to be independent on IFN-γ secretion but dependent on engagement of CD48 present on B cells by NK-expressed CD2 or CD244 [2]. NK cells have also been implicated in modulating the ability of B cells to present antigen to T cells [3]. In the current issue of *JLB*, Sinha and colleagues [4] reveal a new, potential role for NK cells in linking innate immunity with B cell fate. They demonstrate that coincubation of B cells with NK cells enhances the expression of endosomal TLR7 and consequently, TLR7 responsiveness in B cells. Thus, antiviral as well as autoimmune antibody responses may be augmented.

In the opening experiment, the authors compared gene expression profiles of follicular B cells and NK cells purified from murine spleens before and after interaction. To exclude potential B cell transcripts induced by IFN-γ derived from NK cells, cells for microarray experiments were derived from IFN-γ knockout mice. Interestingly, despite the absence of IFN-γ, many of the genes up-regulated in B cells, interacting with NK cells, belonged to the group of the IFN-stimulated gene family. Among the up-regulated B cell genes was TLR7, an endosomal TLR first described as a receptor recognizing ssRNA. Additionally, TLR7 has been found to play an important role in inducing activation of autoantigen-reactive B cells [5]. In the further experiments of the paper, the authors focused their attention on NK-induced augmentation of TLR7 expression in B cells both purified from murine spleens. They confirmed the up-regulation of TLR7 upon NK–B cell interactions by RT-PCR. The latter technique was also used for assessment of IL-6 mRNA as a functional readout of TLR7 up-regulation. In the absence of TLR7 stimuli, the levels of IL-6 mRNA were barely detectable. Therefore, the authors added an imidazoquinoline compound as a TLR7 ligand to the cultures in experiments where IL-6 production was assessed. However, they did not show IL-6 expression at a protein level or IgG secretion in response to the stimulation.

So far, enhanced TLR7 sensitivity in the B cell has mainly been observed in the presence of IFN type I derived from pDCs or from B cells themselves [6, 7] . Although NK cells are not known to be a relevant source of type I IFNs, the authors repeated their experiments using IFNAR$^{0/0}$ mice lacking IFN-α/βR expression and still observed augmentation of TLR7 expression. Therefore, they hypothesized that another class of IFNs, type III or IFN-λ, may mediate the observed effect. IFN-λ—also known as IL-28/29—were identified about 10 years ago and are thought to contribute to antiviral defense at mucosal surfaces [8]. A caveat to their hypothesis is that so far, IRF7 up-regulation, as a measurement of IFN response, has mainly been observed in pDCs and in epithelial cells upon IFN-λ stimulation [9]. Again, here, with the use of an anti-IL-28R antibody, very faint expression of the IL-28R on B cells was observed, which could be enhanced only minimally upon coculture with NK cells. Despite the detection of only low levels of the IL-28R, the addition of anti-IL-28R antibody or addition of antibody against the IL itself to the cultures clearly reduced the expression of TLR7 and the induction of IL-6 production upon addition of the TLR7 ligand by ~60%. In contrast to the present study, where an approximate sixfold increase of IRF7 was observed in B cells upon NK coincubation, Ank et al. [9] observed no IRF7 up-regulation in B cells upon stimulation with high levels of IFN-λ. However, it is conceivable that the observed up-regulation of IRF7 in the work of Sinha et al. [4] was not only a result of IFN-λ-mediated effects but also a result of cell–cell interactions. In fact, the authors also observed reduced levels of TLR7 expression in B cells when cellular contact between NK and B cells was

Abbreviations: IRF7 = IFN regulatory factor 7, pDC = plasmacytoid DC

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disturbed. Thus, TLR7 up-regulation in B cells upon contact with NK cells seems to be mediated by cellular interactions as well as IL-28 stimulation. However, which surface structures are involved in the B–NK cell interaction remains an open question.

In conclusion, the authors suggest that NK-derived IFN-λ provides an alternative pathway of enhancing TLR7 expression in B cells and may play an important role in the induction of autoimmune B cell responses. The authors propose a model in which the enhancement of TLR7 expression by NKs could be the first step for surpassing the threshold for activation of autoreactive B cells upon encounter with the autoantigen, whereupon autoantibodies will be produced (**Fig. 1**). The authors further suggest that consequently, immune complexes consisting of autoantibodies and autoantigen will activate pDCs to produce type I IFN and hence, will provide a positive feedback loop promoting TLR7 expression and resulting in increased autoantibody production. This intriguing hypothesis, however, warrants further investigation in WT mice as well as in autoimmune-prone in vivo models.

Although the data presented by Sinha et al. [4] clearly suggest a role for NK-derived IL-28 in the enhancement of TLR7 induction, some questions remain open. The nature of the other (surface) factors involved in NK–B cell interactions leading to TLR7 augmentation would be of interest. Furthermore, the present study focuses on the impact of NK cell-derived IL-28 on the TLR7 increase in B cells. However, IL-28 is also produced by a variety of other cells, e.g., DCs or epithelial cells, upon stimulation [10]. Are those cells also capable of increasing TLR7 expression in B cells and hence, autoantibody production, and, if yes, to which extent do they contribute to autoimmunity? Another open question is which impact the ratio of NK:B cells may have on the observed effect. In this context, it will be of interest to assess whether the data from the in vitro model, where NK and B cells were cultured at a ratio of 1:2, can be translated into an in vivo model, as the ratio of NK:B cells in the spleen is ~1:30.

Last but not least, it should be borne in mind that all experiments were conducted with murine cells and that the question of whether the present findings in mice can be translated into the human system remains to be clarified. In this context, it has been shown, for instance, that the NK–B cell interaction resulted in Ig production in mice and men but that this was dependent on the interaction of different receptors (CD40–CD40 ligand in humans, CD48–CD2/244 in mice). Nevertheless, several studies suggest an involvement of NK cells in the development of human autoimmune diseases, but their exact role is, so far, not fully understood. Genetical linkage analysis showed that various autoimmune diseases, such as multiple sclerosis or systemic lupus erythematosus, were associated with up-regulation of certain NKR genes. However, there are controversial data about whether NK cells are increased or decreased in the blood of patients suffering from systemic lupus erythematosus. Furthermore, if and to which extent the B–NK cell interaction takes place in human autoimmune diseases, so far, have not been assessed in humans. Interestingly, IFN-λ and its receptor have been shown to be strongly expressed in the epidermis of cutaneous lupus erythematosus

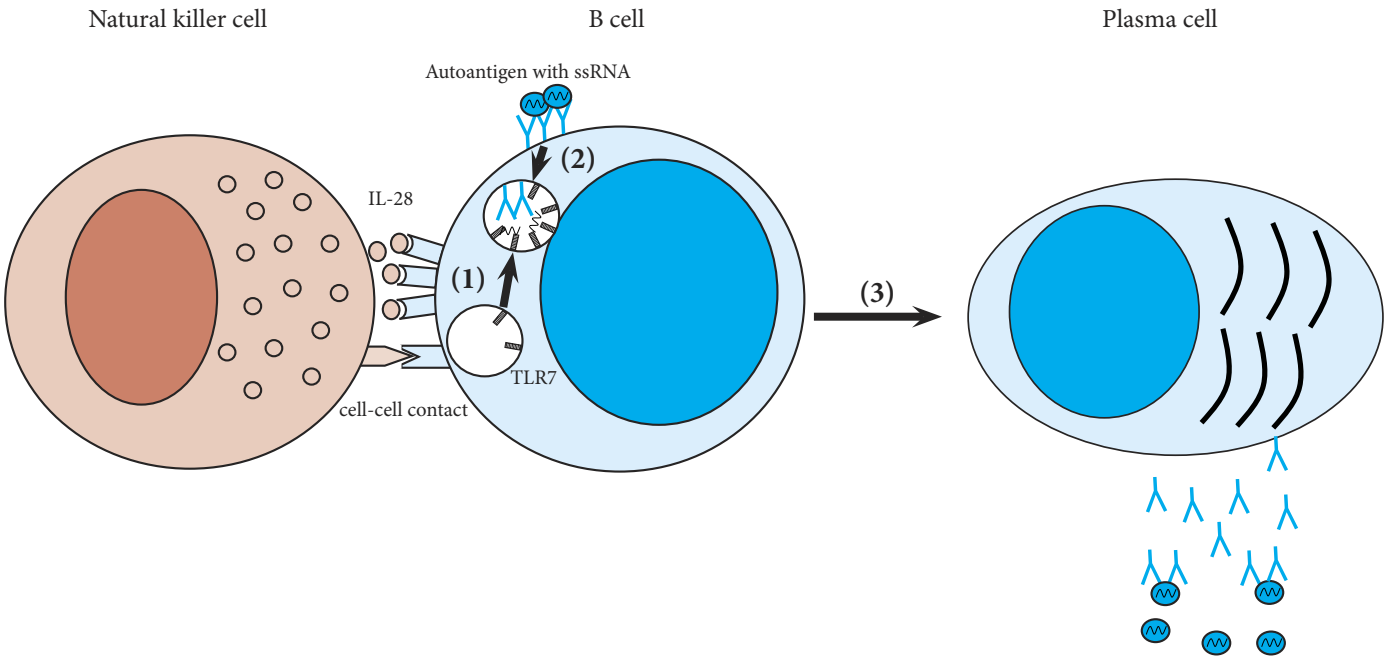

Figure 1. NK cells enhance TLR7 expression in B cells via cellular contact and IL-28 secretion. (1) NK cells enhance endosomal TLR7 expression in B cells by IL-28 secretion and cell–cell contact (2). BCR-internalized autoantigen is recognized by endosomal TLR7, and the costimuation results in (3) plasma cell differentiation and autoantibody production.

lesions by keratinocytes [11]. Therefore, based on the findings of the present study, the questions of whether IFN-λ has a similar effect on human B cells and whether human NK cells derived from autoimmune patients produce IFN-λ and are able to stimulate B cells warrant further investigation.

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KEY WORDS:
TLR-7 · IL-28 · NK-cell · Immunoglobulin · B cell