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Commentary

Natural killer (NK) cells play an important role in host defense against viral infections and malignancy, and their role for regulating other components of the antiviral response is being investigated. In this issue of the *JCI*, Ali et al. examine the mechanisms by which NK cells migrate into the white pulp and mediate suppression of virus-specific T cells. Herein, the authors show that an acute lymphocytic choriomeningitis virus (LCMV) infection induced a potent type I IFN (IFN-I) response that resulted in the expression of chemokine receptor CXCR3 ligands and permitted NK cell trafficking to T cell zones. Collectively, these findings have broad implications for vaccination strategies and warrant further investigation into the transcriptomic profiles of these regulatory NK cells.

Inclusion criteria: how NK cells gain access to T cells

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Natural killer (NK) cells play an important role in host defense against viral infections and malignancy, and their role for regulating other components of the antiviral response is being investigated. In this issue of the *JCI*, Ali et al. examine the mechanisms by which NK cells migrate into the white pulp and mediate suppression of virus-specific T cells. Herein, the authors show that an acute lymphocytic choriomeningitis virus (LCMV) infection induced a potent type I IFN (IFN-I) response that resulted in the expression of chemokine receptor CXCR3 ligands and permitted NK cell trafficking to T cell zones. Collectively, these findings have broad implications for vaccination strategies and warrant further investigation into the transcriptomic profiles of these regulatory NK cells.

NK cell localization

NK cells have been shown to directly lyse T cells (1–3), but the mechanisms by which NK cells control adaptive immunity remain unclear. Mechanistically, NK cell–mediated regulation of antiviral T and B cell responses depends on the perforin pathway, and type I IFN (IFN-I) is critical for NK cell activation and cytolytic activity (3–6). However, NK cells are typically excluded from T cell zones, and the factors regulating their recruitment and entry into the white pulp of the spleen were unknown. In this issue of the *JCI*, Ali and colleagues (7) investigated NK cell–mediated suppression of adaptive-immune responses using two disparate types of viral infections, lymphocytic choriomeningitis virus (LCMV), which induces NK cell–mediated targeting of virus-specific $CD4^+$ T cells, and adenoviral vectors, which weakly induce IFN-I and do not induce NK cell–mediated targeting.

NK cells are classically defined by their cell-surface expression of NK1.1 and NKp46 (mice) or CD56 (human). To quantitatively assess NK cell localization, the authors employed an innovative vascular staining technique to rapidly label NK cells in the red pulp by intravenously injecting an NKp46 antibody. As expected in the absence of infection, NK cells were located primarily in the red pulp. Following LCMV infection, there was a transient increase in the NK cell population in T cell zones. Specifically, there was a three-day window of opportunity wherein the proportion of NK cells within the white pulp increased, and by day 6 after infection, the population was similar to that in uninfected controls (Figure 1). Ali et al. then compared the NK cell population in the white pulp to that in the red pulp and found that phenotypically, these cells expressed similar levels of activating and inhibitory receptors; however, there was a slight reduction in NKG2D (7). This change in NKG2D is intriguing because other groups have shown NK cells can lyse activated T cells vis-à-vis NKG2D and TRAIL (3, 8). Moreover, when comparisons were made between the red and white pulp, the authors found that the NK cell subset distribution and expression of the chemokine receptor CXCR3 were similar. In contrast, granzyme B and CD25 levels were slightly elevated in the NK cell population from the white pulp. Importantly, the researchers also observed an increase in the expression of CXCR3 ligands following LCMV infection in contrast with immunization with adenovirus serotype 5 vector (Ad5).

Ali et al. proceeded to demonstrate a critical role for IFN-I in driving the NK cells into the white pulp. They compared mice that were infected with Ad5 and treated with recombinant IFN-α with mice that were acutely infected with LCMV and treated with function-blocking antibodies against IFN-αβ receptor 1 (IFNAR-1). The addition of IFN-α to Ad5-infected mice led to an increased number of NK cells within the white pulp, whereas inhibition of IFN signaling in the presence of an LCMV infection reduced the number of NK cells in the T cell zones. To address the mechanisms by which NK cells are recruited to the white pulp, the authors conducted a unique set of mixed bone marrow chimera studies using recombination-activating gene (RAG) knockout mice. The authors mixed the donor bone marrow in a manner that resulted in normal expression of CXCR3 in the adaptive immune cells (B cells and T cells), whereas the NK cells were either CXCR3 sufficient or CXCR3 deficient. Ali et al. found that there were fewer CXCR3-deficient NK cells within the splenic white pulp and T cell zones compared with their CXCR3-sufficient counterparts. Importantly, virus-specific T cell responses were similar in NK cell–depleted and CXCR3-deficient NK cells, demonstrating a critical role for CXCR3 in NK cell–mediated regulation of activated T cell responses. To confirm their findings, the authors transferred LCMV-specific T cells into wild-type and CXCR3-deficient mice and examined responses in control and NK cell–depleted hosts. The reduction in LCMV-specific T cells was completely abrogated in CXCR3 knockout hosts; in fact, the numbers were similar to those in NK cell–depleted hosts, demonstrating that NK cells specifically regulate antiviral CD4 responses in a CXCR3-dependent manner (7).

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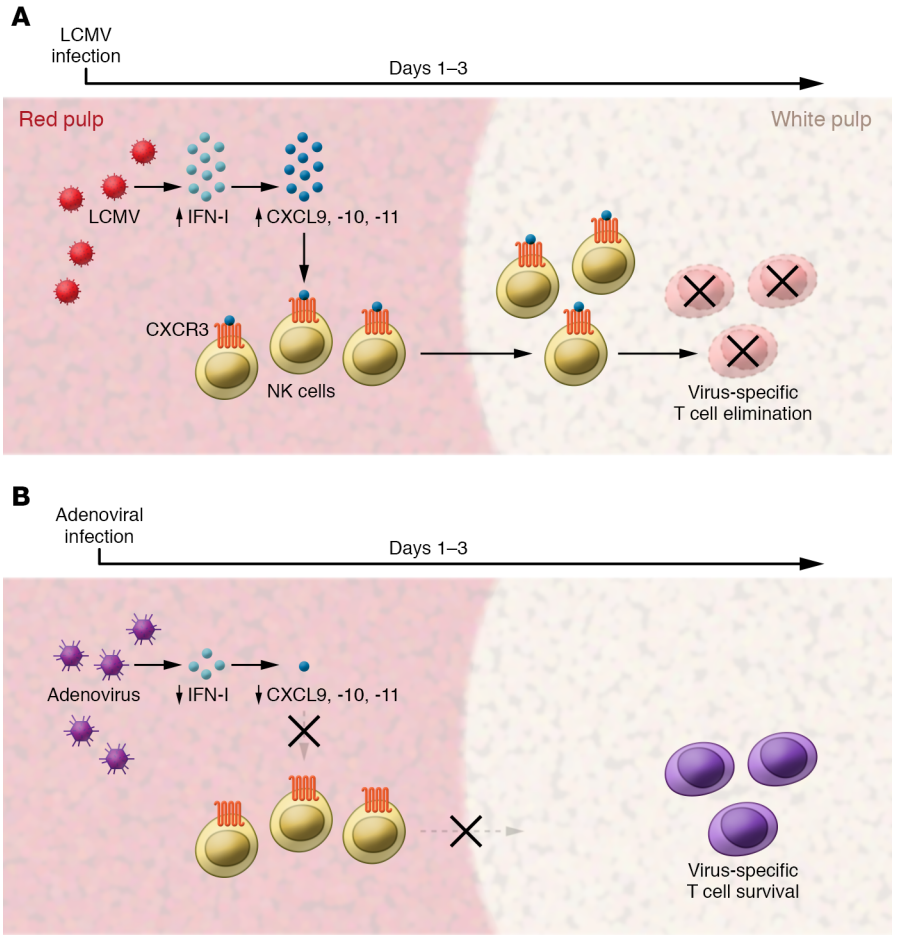

Figure 1. Potent IFN-I responses provide access to regulatory NK cells. (**A**) Viral infections that induce high levels of IFN-I and subsequently lead to the production of CXCR3 ligands allow the transient recruitment of NK cells into the white pulp of the spleen, wherein the NK cells can induce cytolysis of virus-specific T cells. (**B**) Viral infections that induce low levels of IFN-I do not allow NK cells to access T cell zones.

NK cells interact with virus-specific T cells

Work from several groups has shown that NK cells regulate T cell responses in the context of viral infections. For example, following an acute LCVM-Armstrong infection, beige mice, which have defective NK cells, have higher numbers of T cells (9). In a series of pioneering studies, Waggoner et al. conducted experiments using low, medium, and high doses of LCMV clone 13 to interrogate the impact of NK cells on antiviral T cell responses (6). At low viral doses, depletion of NK cells resulted in higher $CD8^+$ T cell responses; however, at medium viral doses, depletion of NK cells protected T cells and permitted a robust T cell response, which was needed to clear the virus. In contrast, at high viral doses, depletion of NK cells resulted in increased pathology and mortality. It was thought that the T cell response required NK cells, and in their absence, lethal T cell–dependent pathology occurred. Previously, Waggoner et al. also showed that following activation with polyinosinic:polycytidylic acid or by infection with LCMV, Pichinde virus, or mouse hepatitis virus, NK cells lysed activated $CD4^+$ T cells. These results suggest that NK cells have a general role in regulating virus-specific $CD4^+$ T cell responses (6). The Ali et al. findings are notable because they demonstrate how NK cells, which are typically excluded from the white pulp, are able to interact with virus-specific T cells (7).

While Ali and authors did not detect many phenotypic differences between the NK cells localized to the red pulp and to the white pulp, they indicate that the two NK cell populations expressed different transcriptomes (7). It is unclear whether cells that gain entry are a unique subset of NK cells or whether the population becomes transcriptionally distinct after entry. In regard to white pulp NK cells, the authors noted that they expressed high levels of granzyme B. As an extracellular enzyme, granzyme B plays a role in cytotoxic lymphocyte extravasation (10). It would be interesting to investigate whether this factor plays a dual role in helping the NK cells to migrate into the white pulp in addition to having a cytotoxic role in regulating the antiviral T cell population.

Clinical implications

While others have previously defined a role for IFN-I responses in regulating the magnitude of antiviral T and B cells responses, Ali et al. elegantly define the parameters for NK cell–mediated regulation of T cell responses (7). This work has several implications for vaccine development. For example, if there is an issue with developing strong T cell memory responses or if there are low antibody titers following vaccination, one could investigate mechanisms for decreasing the initial IFN-I response by decreasing the dose of adjuvant or using inhibitors of CXCR3 or its ligands. Given the strong regulatory role that NK cells have on T cells, it is interesting to speculate on how these findings may be used in other contexts beyond viral infections, such as in autoimmunity. For example, modulation of NK cell activity directly affects $CD4^+$ T cell–mediated autoimmune disease in several mouse models (11–13). Ali et al. demonstrated a key role for CXCR3 ligands in the recruitment of NK cells to T cell zones (7). CXCR3 ligands and CXCL9, -10, and -11 have different binding affinities and can have different functions. Perhaps the selective induction or use of CXCR11 could be leveraged to recruit NK cells and Tregs in order to ameliorate inflammatory disorders or autoimmune disease.

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