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Cutting Edge: Induction of Inflammatory Disease by Adoptive Transfer of an Atypical NK Cell Subset

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Several mouse models of systemic lupus erythematosus, including FcRIIB-KO and TLR7tg mice, develop an expansion of an atypical NK cell subset with functional similarity to cells referred as IFN-producing killer DCs or pre-mature NKS in other systems. In this study, we show that atypical NKS purified from spleens of systemic lupus erythematosus–prone mice, and identified as NK1.1⁺CD11c⁺CD122⁺MHC-II⁺, induce persistent autoimmune disease in an IFN-I– and CD40L-dependent manner when transferred to wild-type mice. A single transfer of 4 × 10⁶ NK1.1⁺ cells from TLR7tg into wild-type induces a 2-wk–long wave of inflammatory cytokines in the serum; a sustained increase in T cell activation and follicular helper cells for the following months; and a progressive expansion of dendritic cells, monocytes, and granulocytes. Furthermore, IL-15 deficiency, which impedes development of NK cells, ameliorates the autoimmune pathology of TLR7tg mice. These results suggest that cells of the NK lineage can develop into cytokine-producing/APCs that affect the priming and progression of systemic autoimmune disease. The Journal of Immunology, 2015, 195: 806–809.

Systemic lupus disease is characterized by a break of immune tolerance to common self-Ags, an IFN signature gene expression, and elevated granulocyte numbers (1, 2). Cytokine-producing cells and APCs such as dendritic cells (DCs) play a central role in the development of autoimmunity due to their ability to activate naive T cells and promote Ab production (3). In addition to DCs, autoimmunity-induced chronic inflammation promotes the appearance of normally rare immune populations with activated phenotype and multiple functionalities that might affect the outcome of disease. For example, we have detected the expansion of cells belonging to the NK cell lineage with an atypical profile in various mouse models of lupus (4). These cells resemble those previously described as IFN-producing killer DC (IKDCs) (5, 6) or premature NKS (7) because they express the surface markers NK1.1 and CD11c and they are efficient cytokine producers and APCs, with high MHC-II expression and cross-presentation ability. NK1.1⁺CD11c⁺ cells from the lupus-prone FcRIIB Yaa and TLR7tg mice have an immature, but activated profile; they are also highly proliferative and survive for months in adoptive transfer experiments (4). Up-to-date IKDCs cells have mostly been linked to tumor environments (8) or TLR7–induced activation (9). In the context of autoimmunity, IKDCs have also been linked to encephalomyelitis (10). We now seek to find whether the type of NK cells that expand in lupus models has in vivo relevance to disease. In this work, we present evidence that NK1.1⁺CD11c⁺ cells from lupus mice can break lymphocyte tolerance and promote long-term myeloid cell expansion when adoptively transferred to wild-type (WT) mice.

Materials and Methods

Mice

The generation of TLR7tg and B6.FcRIIB⁻/⁻ mice as well as IL-15⁻/⁻, IL-18⁻/⁻, IFN-α⁻/⁻, IFN-β⁻/⁻ has been described earlier (11–15). Mice were used at 8–12 wk of age, except in survival studies. Housing at the National Institutes of Health facility met the Instructional Animal Care and Use Committee and National Institutes of Health guidelines.

Adoptive transfer

Transferred NK1.1⁺CD11c⁺ cells were sorted by FACS Aria (BD Biosciences) or purified by a combination of CD11c and NK1.1-positive bead selection (RoboSep; Stemcell Technologies) with comparable results. A total of 4 × 10⁶ cells was injected i.v. per mouse.

Pathology

ELISA was used to measure serum RNA Abs (Immco Diagnostics) and total IgG (Southern Biotechnology). Hematological scores were determined by a Hemavet (Drew Scientific). Serum cytokines were quantified by cytometric bead array (BD Biosciences). For histology, 10% formalin-fixed organs were stained with H&E, and inflammatory scores were as described (16).

Statistical analysis

Statistical significance was determined by the Student t test, one-way ANOVA, and Kaplan–Meier for the survival curve.

Results and Discussion

Adoptive transfer of NK1.1⁺CD11c⁺ cells induces long-term autoreactivity and inflammation

We have previously reported the expansion of a NK subset identified as NK1.1⁺CD11c⁺CD3⁻CD122⁺MHC-II⁺E4BP4⁺Tbet⁺ in autoimmune-prone mice (4). To investigate the role of these cells in vivo, we purified NK1.1⁺ splenocytes from WT and TLR7tg spleens and i.v. injected 4 × 10⁶ cells into untouched WT mice. This represented the transfer of about all the NK1.1⁺ cells from a single TLR7tg spleen into three recipient mice. TLR7tg-derived NK1.1⁺ cells were determined to be...
MHC-II+CD122+CD11c+ in all experiments (Fig. 1A). We also confirmed on at least one experiment that this population was devoi do of T cells, mature NK cells, or plasmacytoid DCs, as they were CD3+CD4+DX5−PDCA−. As control, NK1.1+ from WT mice represented conventional NK cells and were all DX5−CD11c−MHC-II+ (Fig. 1A). Donor populations were purified either by cell sorting TLR7tg NK1.1+CD11c+ and WT NK1.1+CD11c− populations, or by NK1.1-positive bead purification. We obtained the same results regardless of the type of purification. Within 1 wk after adoptive transfer of TLR7tg NK1.1+ cells into WT mice, we detected increased expression of inflammatory cytokines that returned to normal or near normal levels by week 4 (Fig. 1B). At 4 wk, we detected augmented titers of total serum IgG and RNA-specific Abs (Fig. 1C), increased number of activated CD4+ cells (CD4+CD45RBhigh), effector/memory CD4 cells (CD4+CD44+CD62L−), follicular helper T cells (CD4+CXCR5+PD1+), germinal center B cells (B220+GL7+FAS+), activated B cells (B220+CD69+), and CD8+ cells expressing the Ly6 Ags in the spleen of recipient mice that received NK1.1+ cells from TLR7tg spleen (Fig. 1D). Thus, this transfer induced autoreactivity that we measured by the presence of autoantibodies and germinal center–associated lymphocytes. High Ly6A/C expression on CD8s was consistent with increased IFN expression. We also detected a progressive myeloid expansion apparent by 4 wk and maximal 4 mo after the transfer of NK1.1+TLR7tg cells. This expansion included conventional DCs (CD11c−CD11b+), inflammatory monocytes (CD11b+Ly6C+), and granulocytes (CD11b+F4/80Ly6Cint) (Fig. 1E). In comparison, transfer of the same number of WT NK1.1+ did not induce a phenotype in recipient mice (Fig. 1B–E).

The ability of NK1.1+CD11c+ cells to break tolerance upon cell transfer is not unique to cells purified from TLR7tg mice. NK1.1+ cells isolated from B6.FcγRIIB−/−Yaa mice also increased serum levels of inflammatory cytokines (Fig. 1F) and induced lymphocyte activation and myeloid expansion in recipient WT mice (Fig. 1G). This is a rare case of long-term induction of autoimmunity and inflammatory pathology from a single injection of a lupus-associated cell population into a nonlupus-prone recipient. Up-to-date, adoptive transfer of B or T lymphocytes or even serum from lupus mouse models has failed to initiate pathology in WT mice recipients. Only the transfer of DCs has been reported to lead to autoimmunity, but even then it does not induce long-term pathology (17, 18). A notable difference that could explain why our adoptive transfer experiments lead to persistent disease and not in those described for DC transfers, is the long survival times and high proliferative capacity of NK1.1+ cells purified from lupus-prone mice (4).

IL-15 deficiency ameliorates and delays pathology in TLR7tg mice

We tested cytokine requirements for the development of atypical NK cells by breeding TLR7tg mice to IL-15−/−, IL-18−/−, and IFN-α/βR1−/− deficient mice. These cells were completely dependent on...
IL-15, but their expansion was unaltered by IL-18 or IFN-αβR1 deficiencies (Fig. 2A). IL-15 dependency suggests these cells are of the NK lineage (19), and, unlike plasmacytoid DCs, they do not require IFN-I for development. IL-18 has been shown to be important for full activation of mature NK cells (20); therefore, the presence of atypical NK cells in IL-18-deficient TLR7tg implies that these cells do not represent activated mature NKS.

We next characterized disease progression in TLR7tg mice that lacked NK1.1+CD11c+ cells (i.e., bred to IL-15-KO) and compared them with TLR7tg mice that bore mutations that would not affect NK1.1+CD11c+ cell numbers, that is, bred to either IL-18-KO or IFN-αβR1-KO (Fig. 2A). We observed that IL-15 deficiency prolonged the survival of TLR7tg mice (Fig. 2B), and also delayed and ameliorated disease in

**FIGURE 2.** IL-15 deficiency delays the development of autoimmune disease in TLR7tg mice. (A) Numbers of splenic NK1.1+CD11c+ cells in each genotype shown, at least three mice per group. **p < 0.01 (Student t test). (B) Survival curve for TLR7tg, TLR7tg.IL15−/−, and TLR7tg. IFNαβR1−/− mice, 15 mice per group. (C) Glomerular damage in kidney and liver inflammation scores measured, as described in Materials and Methods; mice of the genotype shown were analyzed for number of platelets (D), total serum IgG and anti-RNA IgG Abs (E), spleen weight (F), splenocyte count (G); data collected from three experiments with three mice per group. *p ≤ 0.05 in ANOVA statistical analysis and Kaplan–Meier for survival curve.

**FIGURE 3.** IFN and T cell help requirement in adoptive transfer of TLR7tg NK1.1+ cells. (A–C) Splenic NK1.1+ cells isolated from wild-type (white bar) or TLR7tg (black bar) were injected into IL15−/−, IFNαβR1−/−, or CD40L−/− mice. Mice from recipient injected with NK1.1+ from TLR7tg mice were tested 1 mo after the transfer for serum cytokines (A). All experimental groups were tested for total serum IgG by ELISA (B). FACS analyses were performed on splenocytes of recipient mice 4 wk after the injection (C). Experiments were performed in at least three mice per group and repeated three times. *p ≤ 0.05.
TLR7tg, even at higher extent as deficiency in IFN-αR1 (Fig. 2B–G). One possible mechanism by which atypical NKS might promote lymphocyte activation is through secretion of IFN-γ, a process directed by IL-18 (20). However, IL-18 deficiency did not alter the phenotype of TLR7tg mice, neither their mortality (Fig. 2B), platelet deficiency (Fig. 2D), autoantibody titer (Fig. 2E), lymphocyte activation, nor myeloid expansion (Fig. 2G). The reduction in mortality of TLR7tg with deficiencies in IL-15 or IFN-αR1 could be explained by the lower level of kidney and liver inflammation (Fig. 2C). However, one phenotype that was not reduced in TLR7tg mice with IL-15-, IL-18-, or IFN-αR1 deficiencies was their low level of platelets, causing what was most likely lethal thrombocytopenia even in delayed pathologies (Fig. 2D). This result implies a platelet-intrinsic effect of the TLR7tg that is independent of factors such as IFN or IL-15.

Our analysis of immune functions showed that IL-15 affected all autoimmune and immune activation in TLR7tg mice: RNA autoantibodies (Fig. 2E), spleen size (Fig. 2F), lymphocyte activation, and myeloid expansion (Fig. 2G) were close to WT levels in TLR7tg mice with IL-15 deficiency. IFN-αR deficiency seemed to have only a partial protective effect, whereas IL-18 deficiency did not affect the disease of TLR7tg mice (Fig. 2B–G).

Requirement of IFN-1 and T cell help in the inflammatory effect of NK1.1+CD11c+ cells

To test functional requirements for the inflammatory function of TLR7tg NK1.1+ cells, we transferred them into IL-15–, IFN-αR1–, or CD40L-deficient recipient mice. We showed above that IL-15 was essential for development and expansion of NK1.1+CD11c+ cells in TLR7tg mice (Fig. 2A). However, IL-15 in the recipient was dispensable in the development of inflammatory phenotype induced by the transfer of NK1.1+ cells from TLR7tg mice (with the caveat that donor cells could be using IL-15 in autocrine fashion). Transferring TLR7tg NK1.1+ cells into IL-15–deficient recipients resulted in elevated serum cytokines (Fig. 3A), IgG (Fig. 3B), lymphocyte activation, and myeloid cell numbers (Fig. 3C). In contrast, IFN-αR deficiency had the opposite effect: IFN-αR was dispensable for development of atypical NKS in TLR7tg mice (Fig. 2A), but this deficiency in recipient mice prevented all phenotypes normally induced by the transfer of TLR7tg NK1.1+ cells, that is, the cytokine expansion (Fig. 3A), serum IgG (Fig. 3B), lymphocyte activation, or myeloid expansion (Fig. 3C). Finally, we determined the requirement for T cell help by performing the adoptive transfer of TLR7tg NK1.1+ cells into CD40L-deficient recipients: this resulted in no autoimmunity, as measured by serum cytokines, serum IgG, or activated CD4+ cells (Fig. 3). However, CD40L deficiency allowed the inflammatory monocyte (CD11b+Ly6C(high) and granulocyte (CD11b+Ly6C(−) expansion detected upon transfer of TLR7tg NK1.1+ cells (Fig. 3C). Overall, these results suggest that the phenotype induced by the transfer of atypical NK cells is fully IFN-1 dependent, and that T cell help is required for the increase in serum inflammatory cytokines, but not for the myeloid expansion.

Overall, our findings point toward a new role for immature NKS that expand and become activated in chronic inflammatory conditions. The appearance of these cells in autoimmune disease could exacerbate the phenotype and lead to a progressively more severe outcome. NK cells have been linked to human systemic lupus erythematosus (SLE) in various studies, although there is no information in reference to immature NK populations. NK Ig-related receptor/HLA genotype has been correlated with autoimmune risk (21), and a lower number and impaired function of NK cells was reported in SLE patients compared with non-autoimmune individuals (22). An appealing theory is that the low level of mature NK cells in SLE patients represents a developmentnal blockade at the immature stage, and that might correlate with the appearance of the type of immature cells that we describe in these studies.

Disclosures

The authors have no financial conflicts of interest.

References


