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MULTI-AUTHOR REVIEW

Editorial overview

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Natural killer (NK) cells are cytotoxic lymphocytes of the innate immune system that play an important role in the immune system's defense against viral infections and tumors. Harnessing the destructive power of NK cells against tumors and virally infected cells may be promising for the development of new immunotherapeutic approaches. More than three decades after the discovery of NK cells [1–4] many aspects of their development and function are still poorly understood and subject of ongoing research. The reviews assembled in this issue highlight recent developments in this rapidly changing field.

One of the fundamental issues in NK cell research has been to unravel how NK cells discriminate between 'innocuous-self' and 'dangerous-self'. NK cells are equipped with immune recognition receptors constantly surveying 'self' cells for pathological and potentially harmful changes, e.g., as a consequence of microbial infection or malignant transformation. In contrast to other innate immune cells (e.g., myeloid cells), NK cells usually do not directly perceive bacterial or viral molecules. Instead, they indirectly sense infections by recognizing changes in cell surface molecules on infected cells. For this surveillance of cellular integrity, NK cells employ receptor systems with opposing qualities: inhibitory receptors and activating receptors. NK cell activity is determined by the integration of activating and inhibitory inputs received upon interaction with a given target cell allowing quantitative and qualitative tuning of NK cell responses. Most inhibitory receptors interact with class I MHC molecules and downregulation of class I MHC, which is frequently observed for virally infected cells and tumors, releases NK cells from inhibition. NK cell-mediated killing of MHC class I (low) cells was the first model to explain how NK cells are activated and has been termed 'missing-self recognition' [5]. Loss of inhibition turned out to be a mode to unleash NK cells but, only more recently, research into activating receptors and their ligands has revealed mechanisms that directly account for NK cell activation. It is now widely accepted that some of the activating receptors recognize stress-inducible cellular ligands. Expression of these ligands, which are cellular 'self' proteins, is tightly regulated and often absent from normal cells. Various forms of cellular stress (including viral and bacterial infection, tumor transformation) have been shown to lead to the up-regulation of such ligands that activate NK cells. This mode of target cell recognition relies on the detection of stress-inducible 'self' molecules (i.e., 'induced-self recognition') and is fundamentally different from the way myeloid cells recognize bacterial or viral 'non-self' [6, 7].

The paradigm of 'induced-self recognition' was brought forward in the context of studies of the activating immunoreceptor NKG2D and its stress-induced MHC class I-related ligands. Here, Polic and colleagues comprehensively review a decade of research on NKG2D and discuss recent developments shedding light on the role of NKG2D in viral infections, tumor surveillance, and pathogenesis of autoimmune diseases. The recent availability of NKG2D-deficient mice has opened new prospects for NKG2D research and provided evidence that NKG2D may also

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influence NK cell development as illustrated by data from the authors' laboratory.

Apart from NKG2D, natural cytotoxicity receptors (NCRs) NKp46, NKp44, and NKp30 represent major activating receptors of NK cells. However, in contrast to NKG2D, cellular ligands of the NCRs have remained elusive. Here, Baratin and colleagues refer to the recent identification of the B7 family member B7-H6 as a ligand of NKp30 and present a wealth of basic information on evolutionary and structural aspects of NKp30 and B7-H6 genes and proteins. Eventually, they also refer to their intriguing observation that expression of B7-H6 so far has only been detected on malignant cells raising the possibility of a unique function of the NKp30/B7-H6 axis in the immunosurveillance of tumors.

Recently, it has become clear that NK cells undergo an education process required for the development of a functional and self-tolerant NK cell pool [8–10]. Various mechanistic models have been proposed to explain this education process ('licensing', 'arming', 'disarming'). All of these models converge on the view that the interaction of inhibitory NK cell receptors with 'self' class I MHC molecules is a requirement to acquire or maintain NK cell function. Thus, inhibitory receptors do not only play a role for NK cell/target cell interactions but are also crucial for generating a functional and 'self'-tolerant NK cell repertoire. It turned out that inhibitory NK cell receptors cannot only interact with class I MHC molecules on target cells (i.e., *trans* interaction) but also bind MHC molecules present on the NK cell itself (i.e., *cis* interaction). Held and Mariuzza summarize the distinct biological roles of ligand binding in *cis* and *trans* by inhibitory NK cell receptors and discuss these findings in the broader context of other receptor systems that use *cis* and *trans* interactions to convey distinct signals. They also highlight the structural requirements allowing a given receptor to access its ligand both on a target cell and within its own membrane. Finally, the important question is discussed of how the cell can discriminate between *cis* and *trans* signals and how they may lead to distinct functional outcomes.

Insights into NK cell function and development from human genetics are presented by Bryceson, Ljunggren, and Wood. Naturally occurring genetic defects in humans affecting NK cells are rare, but have provided important insights into various aspects of NK cell biology. In particular, the authors highlight genetic defects that have advanced our understanding of the molecular machinery orchestrating NK cell cytotoxicity as well as vital contributions of NK cells to immunosurveillance emerging from severe infections associated with mutations in genes governing NK cell reactivity.

For a long time, NK cells were believed to be 'natural killers' that follow a cell-autonomous activation program and do not depend on additional signals for their activation. Over the last few years, this paradigm has been challenged because it has become apparent that NK cells require interactions with myeloid cells, in particular dendritic cells (DC) in secondary lymphoid organs to effectively fight infections and tumors. Two reviews highlight important advances of our understanding of how DC 'prime' or sensitize NK cell function. Barreira da Silva and Münz focus on the interaction between human NK cells and DC. They summarize the myeloid cell-derived signals identified to date that are involved in instructing NK cell functionality. In addition, they provide a new concept of how these signals are structured for different 'decisions' on the level of the NK cell/DC synapse. In addition to the inhibitory and activating synapse, they introduce the regulatory synapse that allows activated DC to 'prime' or sensitize NK cells while being spared from NK cell attack. Braud and colleagues give an overview of the activation of mouse NK cells. In addition to DC interaction via IL-15-mediated signals, they summarize new data assigning an important role to T cell-derived IL-2 for NK cell activation likely in scenarios when IL-15 is limiting.

The transcriptional programs that instruct NK cell development and NK cell effector fate decisions are still poorly defined. Martin-Fontecha and colleagues discuss recent advances in understanding the transcriptional networks required for NK lineage decisions and effector functions. This review also highlights the recent discovery of the transcription factor E4bp4 (or Nfil3) that is required for NK cell development but redundant for the development of all other known innate and adaptive lymphocyte lineages. E4bp4 may constitute the NK lineage-defining transcription factor [11, 12]. Very recently, NK cell receptor (NKR)-expressing innate lymphoid cells with unusual features have been discovered. Those subsets were identified in mice and humans and the most visible of these subsets co-expresses NKRs and the orphan transcription factor RORγt and is an important innate source of IL-22 [13–17]. Diefenbach and Mortha discuss recent findings regarding the developmental relationship of $NKR^{+}ROR\gamma t^{+}$ cells with NK cells and other innate lymphoid cells such as lymphoid tissue inducer (LTi) cells. They also propose a unifying concept to classify these and other novel innate lymphocyte subsets expressing NKRs.

The various facets of NK cell research highlighted by these reviews continue to fascinate immunologists coming from different backgrounds into this flourishing field of cytotoxic innate lymphocytes and their role in health and disease of mammals. The following years can be expected to provide new and exciting insights into the transcriptional networks regulating cell fate decisions of innate lymphocytes, into molecules recognized by activating NK receptors, into rules assuring self-tolerance of NK cells, into

adaptive features of NK cell responses as well as into the newly described innate lymphocyte subsets and their role in tissue homeostasis. Research into the various aspects of NK cell biology may allow to harness the power of NK cells for the immunotherapy of tumors and in the context of bone marrow transplantation for the cure of cancer.

References

1. Kiessling R, Klein E, Wigzell H (1975) "Natural" killer cells in the mouse. I. Cytotoxic cells with specificity for mouse Moloney leukemia cells. Specificity and distribution according to genotype. Eur J Immunol 5:112–117
2. Kiessling R, Klein E, Pross H, Wigzell H (1975) "Natural" killer cells in the mouse. II. Cytotoxic cells with specificity for mouse Moloney leukemia cells. Characteristic of the killer cell. Eur J Immunol 5:117–121
3. Herberman RB, Nunn ME, Lavrin DH (1975) Natural cytotoxic reactivity of mouse lymphoid cells against syngeneic and allogeneic tumors. I. Distribution of reactivity and specificity. Int J Cancer 16:216–229
4. Herberman RB, Nunn ME, Holden HT, Lavrin DH (1975) Natural cytotoxic reactivity of mouse lymphoid cells against syngeneic and allogeneic tumors. II. Characterization of effector cells. Int J Cancer 16:230–239
5. Karre K, Ljunggren HG, Piontek G, Kiessling R (1986) Selective rejection of H-2-deficient lymphoma variants suggests alternative immune defense strategy. Nature 319:675–678
6. Diefenbach A, Raulet DH (2001) Strategies for target cell recognition by natural killer cells. Immunol Rev 181:170–184
7. Medzhitov R, Janeway CA Jr (2002) Decoding the patterns of self and nonself by the innate immune system. Science 296:298–300
8. Kim S et al (2005) Licensing of natural killer cells by host major histocompatibility complex class I molecules. Nature 436:709–713
9. Fernandez NC, Treiner E, Vance RE, Jamieson AM, Lemieux S, Raulet DH (2005) A subset of natural killer cells achieves self-tolerance without expressing inhibitory receptors specific for self-MHC molecules. Blood 105:4416–4423
10. Raulet DH, Vance RE (2006) Self-tolerance of natural killer cells. Nat Rev Immunol 6:520–531
11. Gascoyne DM et al (2009) The basic leucine zipper transcription factor E4BP4 is essential for natural killer cell development. Nat Immunol 10:1118–1124
12. Kamizono S et al (2009) Nfil3/E4bp4 is required for the development and maturation of NK cells in vivo. J Exp Med 206:2977–2986
13. Sanos SL, Bui VL, Mortha A, Oberle K, Heners C, Johner C, Diefenbach A (2009) RORγt and commensal microflora are required for the differentiation of mucosal interleukin 22-producing NKp46+ cells. Nat Immunol 10:83–91
14. Satoh-Takayama N et al (2008) Microbial flora drives interleukin 22 production in intestinal NKp46+ cells that provide innate mucosal immune defense. Immunity 29:958–970
15. Luci C et al (2009) Influence of the transcription factor RORγt on the development of NKp46+ cell populations in gut and skin. Nat Immunol 10:75–82
16. Cupedo T et al (2009) Human fetal lymphoid tissue-inducer cells are interleukin 17-producing precursors to RORC+ CD127+ natural killer-like cells. Nat Immunol 10:66–74
17. Cella M et al (2009) A human natural killer cell subset provides an innate source of IL-22 for mucosal immunity. Nature 457:722–725