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Jonjić, Stipan

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Commentary

Functional plasticity and robustness are essential characteristics of biological systems: Lessons learned from KLRG1-deficient mice

Stipan Jonjic

Department of Histology and Embryology/Center for Proteomics, Faculty of Medicine, University of Rijeka, Rijeka, Croatia

Killer cell lectin-like receptor G1 (KLRG1) receptor is considered to be a marker of terminally differentiated NK and T cells and is strongly induced by viral and other infections. KLRG1 is a C-type lectin-like inhibitory receptor, which interacts with members of the cadherin family of molecules leading to the inhibition of T- and NK-cell function. A study in this issue of the *European Journal of Immunology* addresses the role of KLRG1 in the maturation and differentiation of NK and T cells *in vivo*. Using KLRG1-deficient mice generated by homologous recombination, the study reveals that KLRG1 is dispensable for NK- and $CD8^+$ T-cell differentiation and function *in vivo*. This interesting finding is discussed in this *Commentary* in light of the plasticity and robustness of immune response mechanisms.

Key words: KLRG1 · KO mice · NK cells

See accompanying article by Gründemann *et al.*

Killer cell lectin-like receptor G1 (KLRG1) is an inhibitory receptor of the C-type lectin-like family, which contains an ITIM in its cytoplasmic domain [1, 2]. It is expressed on certain subsets of NK and T cells that have an effector or effector-memory phenotype [3, 4]. KLRG1 is generally used as a marker of terminally differentiated NK and T cells and is strongly induced by viral and other infections [3]. KLRG1 expression by NK cells after viral infection inversely correlates with NK cells' ability to produce IFN-γ [5]. Experimental evidence indicates that the interaction of KLRG1 with its cognate ligand, E-cadherin [6] can result in the inhibition of T- and NK-cell function [7, 8]. It has been proposed that KLRG1 raises the activation threshold of NK and T cells, thereby attenuating effector responses and reducing the risk of autoreactivity [9, 10]. However, these KLRG1–E-cadherin interactions appear to be very weak and may be functionally significant only under conditions where multimeric receptor/ligand complexes are formed [8]. It has been suggested that NK cells bearing KLRG1 can monitor the expression of several cadherins on target cells, resulting in MHC-independent missing self-recognition [11]. The functional role for KLRG1 in primary human $CD8^+$ T cells was recently demonstrated by blocking KLRG1 signaling using antibodies against E-cadherin. This resulted in a significant enhancement of Akt phosphorylation and TCR-induced proliferative activity of senescent T cells [12].

Although the idea of KLRG1 being a marker of terminally differentiated lymphocytes makes sense, the majority of conclusions reached so far stem from *in vitro* data, and major insights remain to come from *in vivo* studies. To address the role of KLRG1 in the maturation and differentiation of NK and T cells *in vivo*, Gründemann *et al.* [13] in this issue of the *European Journal of Immunology* present very interesting data on the generation and characterization of KLRG1-deficient mice obtained by homologous recombination. By elegantly designing a well-controlled set of experiments in several relevant infection models, the authors show

Correspondence: Professor Stipan Jonjic
e-mail: jstipan@medri.hr

that KLRG1 deficiency affects neither the differentiation nor the function of NK or $CD8^+$ T cells. In addition, $KLRG1^{-/-}$ mice generate a normal antiviral $CD8^+$ T-cell response during both the acute and memory phase of infection. Thus, despite the fact that KLRG1 is abundantly expressed on anti-viral effector $CD8^+$ T cells, KLRG1 deficiency does not affect the induction of antigen-specific $CD8^+$ T cells after viral infection. Adoptively transferred T cells from $KLRG1^{-/-}$ and WT mice proliferate to the same extent in recipient mice after LCMV infection, and give rise to similar numbers of memory T cells. The expression of typical markers on effector and memory T cells and their capacity to secrete IFN-γ and IL-2 after antigen stimulation was also unaffected. In agreement with previous results, the authors also show that KLRG1 ligand expressed on target cells can inhibit the reactivity of NK cells that are derived from transgenic mice overexpressing KLRG1, but not NK cells derived from either $KLRG1^{-/-}$ or WT mice. The explanation for this finding is that the KLRG1–ligand interaction is very weak, and hence a strong activating signal can overcome the inhibition induced by KLRG1. Surprisingly, NK cells from $KLRG1^{-/-}$ mice are more strongly activated after co-culture with cells expressing E-cadherin compared with control cells that do not express this ligand, perhaps suggesting the involvement of an activating receptor.

What do gene KO mice lacking this seemingly important receptor but showing no expected phenotype teach us? Is it really possible that KLRG1 does not play an important role in NK-cell and T-cell biology? When drawing conclusions from such findings, one has to bear in mind that the immune system, like any other physiological system, is able to compensate even for the lack of physiologically important functions. There are many examples of KO mice in which the targeted function is not affected, although one might have expected dramatic changes based on the known function of the gene (for review see [14]). The immune system is in fact one of the best examples of the importance of functional redundancy regardless of whether it is compensation by other homologous genes, or functional compensation at other levels of biological organization. In a biological context, functional redundancy is extremely important when it comes to dealing with infections, as it is essential that the elimination of invading microbes does not rely on a single mechanism. This is why it does not come as a surprise that the deletion of only one receptor or even a cell type involved in immune control will not necessarily result in an obvious phenotype. As Gründemann *et al.* rightly emphasize, the function of NK and $CD8^+$ T cells is regulated *via* a number of activating and inhibitory receptors and the lack of only one inhibitory receptor will not necessarily cause functional alterations.

As an example of the robustness of immune control, it is important to point out the recently published data on NKG2D-KO mice [15, 16]. NKG2D is a dominant activating receptor on NK cells and a co-stimulatory receptor on $CD8^+$ T cells. It recognizes several different ligands induced by cellular stress or transformation. It has been shown that NKG2D plays an important role in the control of MCMV infection *in vivo*; its importance is additionally illustrated by the fact that both human and mouse CMV encode both proteins and miRNA, which downregulate the expression of NKG2D's ligands [17]. Thus, it makes perfect sense to assume that NKG2D-KO mice might have at least some difficulties in the early control of MCMV infection; however, it turns out that KO mice control MCMV infection equally well, or even better in some tissues, than control mice. Although the mechanism underlying this finding has not yet been characterized, the results highlight the plasticity of immune control mechanisms. Not all functions of NKG2D, however, can be compensated for. As reported by Guerra *et al.* [16], NKG2D is critical for immunosurveillance of epithelial and lymphoid malignancies. In another study, the deletion of another NK-cell-activating receptor, NKp46 (NCR1), also did not result in any changes in the maturation and differentiation of NK cells [18]; however, as NKp46 binds to influenza hemaglutinin, the KO mouse is very sensitive to the influenza virus. An interesting phenotype of this mutant mouse strain was recently described, suggesting that NKp46 may be involved in progression of type 1 diabetes by interfering with unexpected NK-cell-mediated recognition of pancreatic beta cells [19], thus indicating that situations may be more complicated than first assessed.

To further emphasize the plasticity and robustness of the immune response, I will give a few additional examples. In early studies, before the era of KO mice, the depletion of T-cell subsets by specific monoclonal antibodies in thymectomized mice was used to create mice lacking a particular type of cells. Such animals were useful tools for elucidating the role of individual subsets in the control of virus infection or other type of immune response. Using the model of MCMV infection, some light was shed on the role of T-cell subsets in virus control [20]. Until then, the results obtained by adoptive transfer of primed T cells into immunodeficient syngeneic recipients clearly demonstrated that $CD8^+$ T cells were essential for virus control, whereas adoptively transferred $CD4^+$ T cells were neither able to reduce the virus titer, nor were they required for antiviral activity of $CD8^+$ cells [21]. Therefore, it could be expected that the depletion of $CD8^+$ cells would compromise the ability of these mice to cope with CMV infection, whereas the depletion of $CD4^+$ cells would have a less significant impact on virus control. The results, however, turned out to be quite the opposite of what was expected, as mice lacking $CD8^+$ T cells cleared the virus with the same kinetics as normal, fully immunocompetent animals [20, 22]. It has also been shown that in the absence of $CD8^+$ cells, virus control is compensated for by a $CD4^+$-cell-dependent mechanism. Therefore, although $CD8^+$ cells are the dominant effector cells in the control of MCMV infection, they are not indispensable, at least according to this particular experimental setting. Moreover, although $CD4^+$ cells derived from immune mice were unable to mediate a protective function after adoptive transfer, depletion experiments in thymectomized mice revealed an, until then, unknown function of this subset that could not be compensated for by $CD8^+$ or any other cells [22]. This function was deduced, as a persistent infection in acinar glandular epithelial cells of salivary glands occurred in CD4-depleted mice and because salivary glands are the major source of CMV for horizontal spread. Thus, it could be concluded that $CD4^+$ cells are essential for this function.

There are many other examples in physiology and cell biology, which show successful compensation for a missing gene and its function or even for entire cell subsets. The effect of some genes might be evident only upon inactivation of another gene. Genetic robustness may also depend on the mouse strain used, as specific alleles of genes present in certain inbred mouse strains have been shown to either partially or completely mask the effects of genetic mutations. Thus, the KLRG1 inactivation in another mouse strain might eventually uncover other, so far unknown, functions of this receptor.

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Abbreviation: **KLRG1:** killer cell lectin-like receptor G1

Full correspondence: Professor Stipan Jonjic, Department of Histology and Embryology/Center for Proteomics, Faculty of Medicine, University of Rijeka, B. Branchetta 20, 51 000 Rijeka, Croatia
Fax: +385-51-651-176
e-mail: jstipan@medri.hr

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