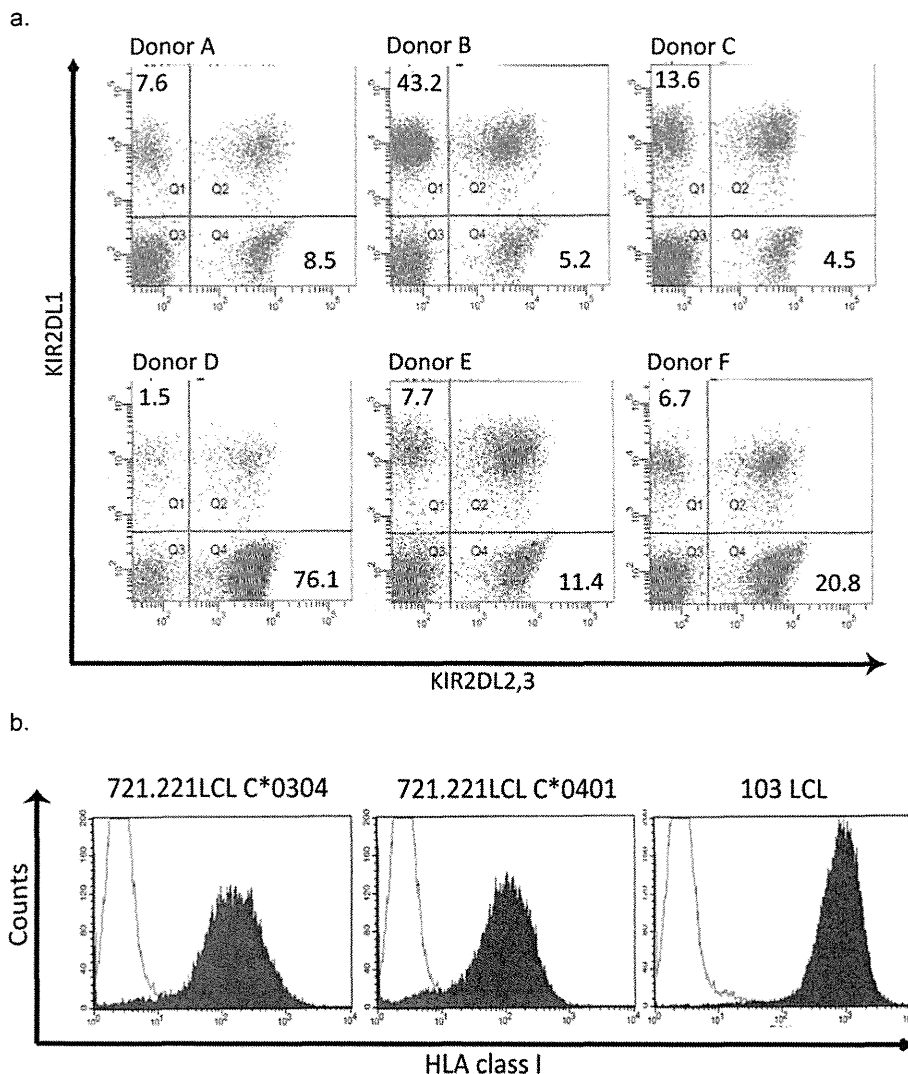

Figure 3: KIR2DL and HLA class I expression in NK cells and target cells. (a) NK cells were purified from a whole blood sample from a healthy donor as described in Materials and Methods. The cells were separated into either KIR2DL2/3+ / KIR2DL1- (KIR2DL2/3+ NK cells) or KIR2DL2/3- / KIR2DL1+ (KIR2DL1+ NK cells) population by FACS sorting. The representative staining of NK cells before sorting in each subject is shown. The value in each quadrant represents the percentages of NK cell subpopulations. (b) Expression of HLA class I on target cells, 721.221LCL HLA-C*0304, 721.221LCL HLA-C*0401, and 103-LCL is shown.

ADCC by rituximab in addition to the natural killing was observed in a significant manner, and HLA-C-uncoupled KIR consistently enhanced the total killing activity consisting of natural killing and rituximab-mediated ADCC. In Figure 4d, the data from all donors were pooled and compared the NK cell killing of HLA-C-coupled KIR with uncoupled (Fig. 4d upper two panels), which shows higher cytotoxicity in HLA-C-uncoupled KIR in both natural killing (IgG) or rituximab-mediated ADCC. Next, 103-LCL cells, which have a homozygous group of HLA-C (C1/-), were used as another target (Fig. 4c).

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In this experiment, enhancement of natural killing by NK cells with C1 group-uncoupled KIR was not observed, whereas rituximab-mediated ADCC was enhanced by HLA-C-uncoupled KIR-bearing NK cells from three out of six donors. Interestingly, all the three donors whose NK cells showed enhancement by HLA-C-uncoupled KIR had C1/C2 groups of HLA-C (Fig. 4c, donor A, B and C).

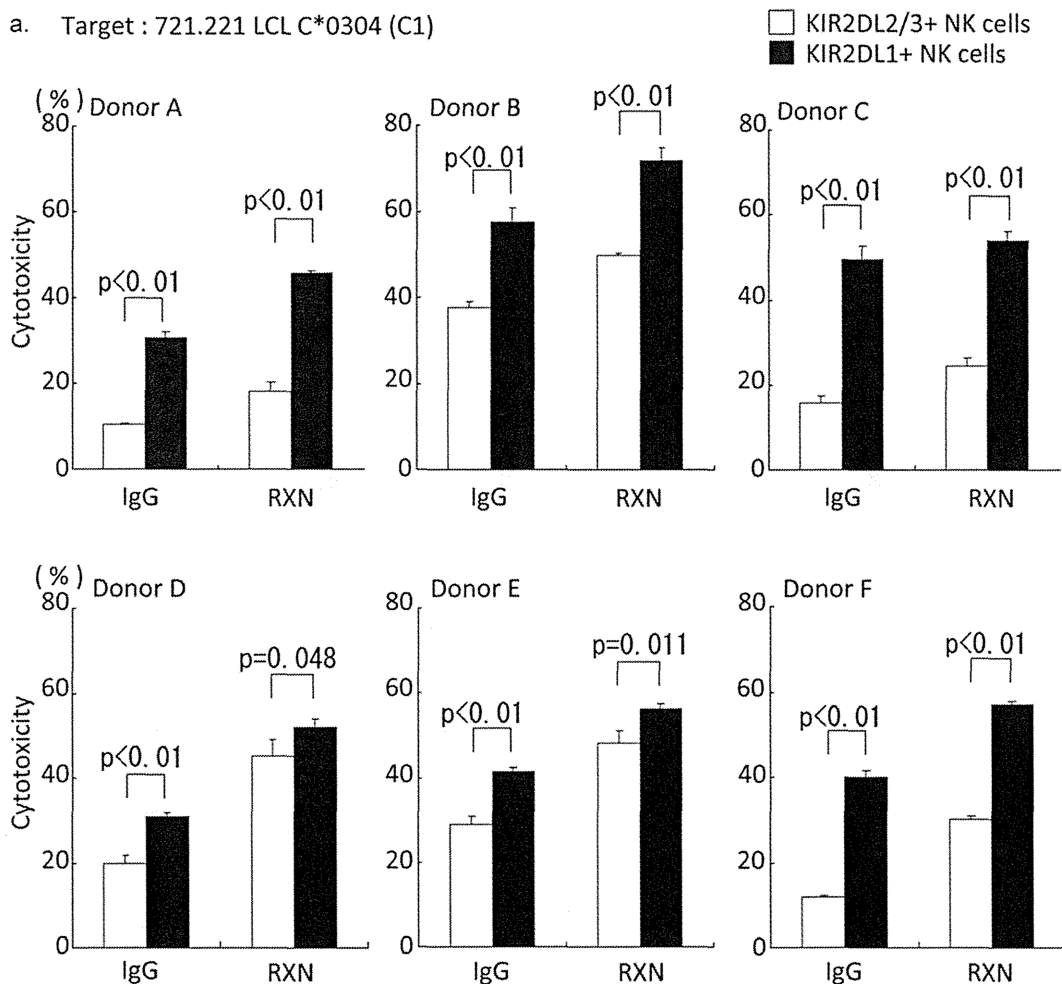

Figure 4: The effect of HLA-C-uncoupled KIR on rituximab-mediated ADCC using purified NK cells. KIR2DL2/3+ and KIR2DL1+ NK cells from healthy volunteers were separately incubated with 721.221LCL HLA-C*0304 (a), 721.221LCL HLA-C*0401 (b), and 103-LCL (c) target cells with 10 µg/ml RXN or control IgG1γ antibody for 5 hours at 37°C, 5% CO_2. The E:T ratio was 5:1. Mean cytotoxicity ± SD of triplicate samples is shown. Data are representative of at least two independent experiments. (d) Comparison of NK cell killing between HLA-C-coupled KIR and HLA-C uncoupled KIR. The data in Figures 4a, b, or c were pooled in every target cell and reanalyzed. In 721.221LCL HLA-C*0304 target cell, HLA-C-coupled KIR and -uncoupled KIR are KIR2DL2/3 and KIR2DL1, respectively; in 721.221LCL HLA-C*0401, HLA-C-coupled KIR and -uncoupled KIR are KIR2DL1 and KIR2DL2/3; and in 103-LCL, HLA-C-coupled KIR and -uncoupled KIR are KIR2DL2/3 and KIR2DL1. (e) The effect of license status on NK cell killing. The results of 721.221LCL HLA-C*0304 target cell in Figure 4a, and 103-LCL in Figure 4c, were reanalyzed, merging the killing of KIR2DL1+ NK cell into licensed or unlicensed categories. "Licensed NK cells" are KIR2DL1+ NK cells from donors A, B and C; "Unlicensed NK cells" are KIR2DL1+ NK cells from donors D, E and F.

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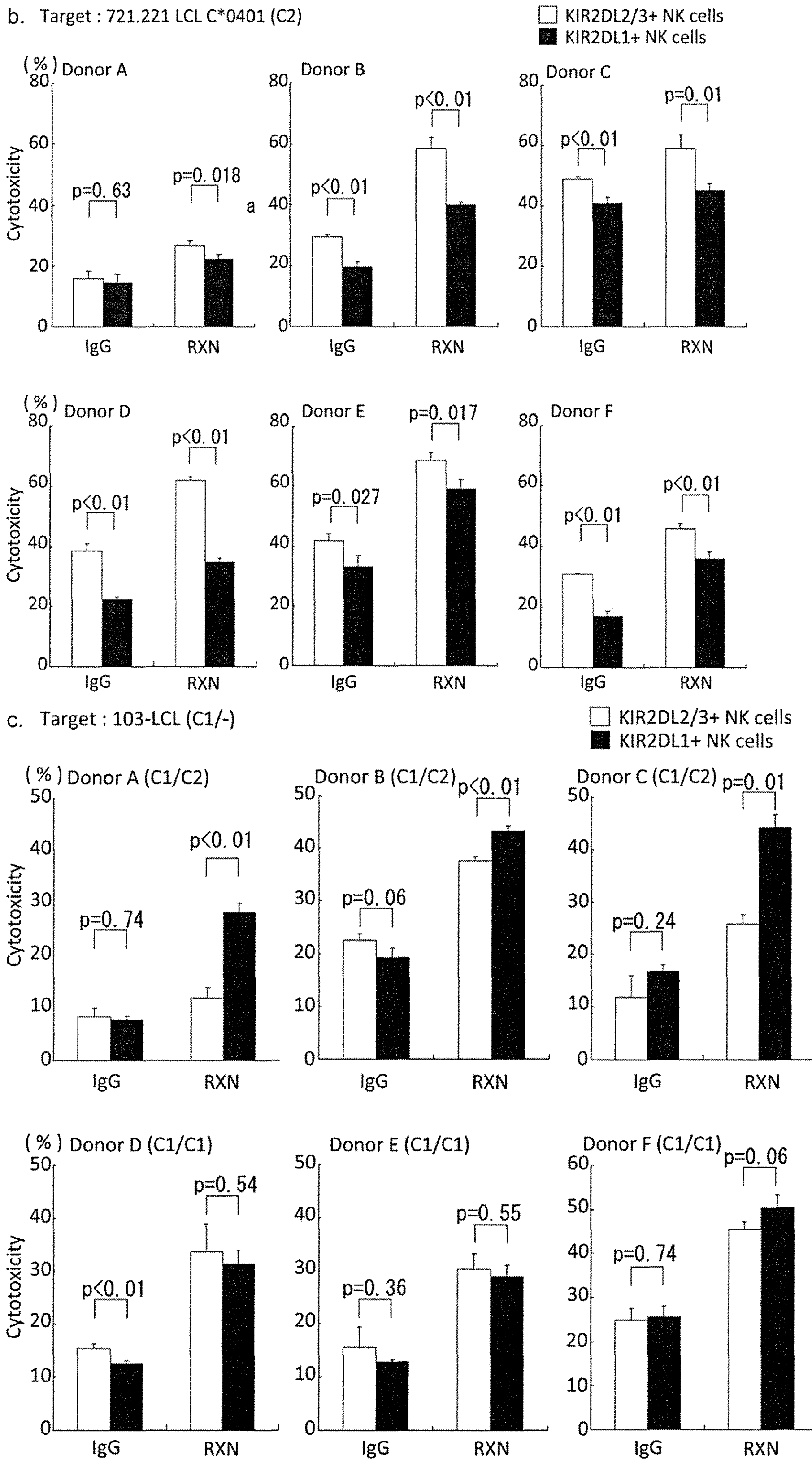

Figure 4: (*Continued*).

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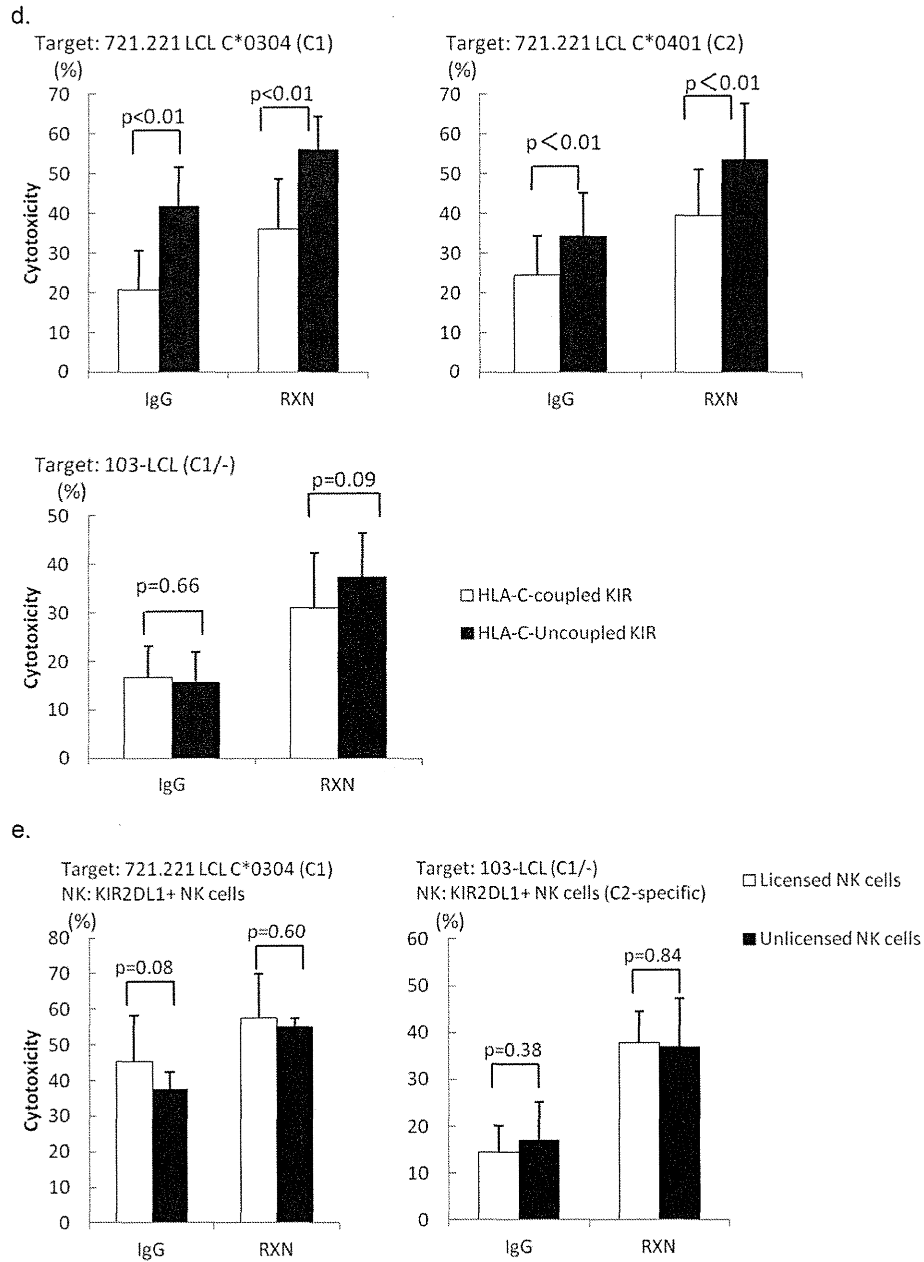

Figure 4: (*Continued*).

In contrast, such ADCC enhancement was not observed with NK cells prepared from all the three subjects having C1/C1 group of HLA-C (HLA-C-matched donors) (Fig. 4c, donor D, E and F). When the data were pooled, the natural killing and ADCC of 103-LCL cells was not different significantly between HLA-C-coupling statuses (Fig. 4d lower panel).

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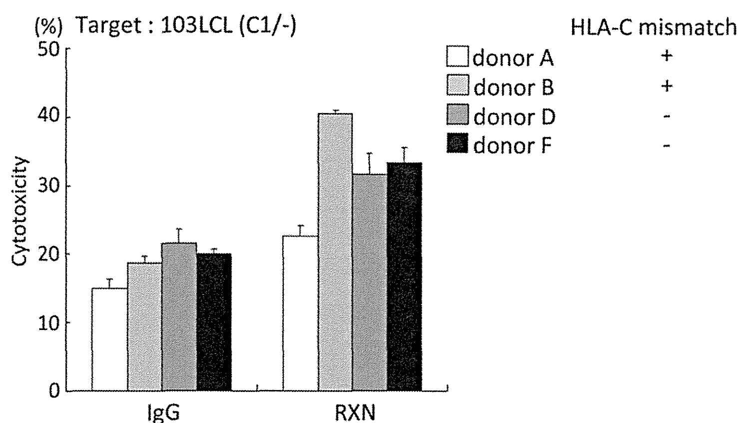

Figure 5: The effect of HLA-C mismatch between donors and target cells on rituximab-mediated ADCC using unsorted NK cells. Unsorted NK cells from healthy donors (donors A and B; HLA-C heterozygous for C1 and C2, donors D and F; homozygous for C1) were incubated with 103-LCL target cells in the presence of 10 μg/ml RXN or control IgG1γ antibody for 5 hours at 37°C, 5% CO_2. The E:T ratio was 5:1. Mean cytotoxicity ± S.D. of triplicate samples was measured. Data are representative of two independent experiments.

Although the two target cells, i.e., 721.221LCL and 103-LCL showed some inconsistent results, experiments using both target cells commonly led to the conclusion that HLA-C-uncoupled KIR enhances rituximab-mediated ADCC by NK cells, at least if prepared from HLA-C-mismatched donors. The impact of HLA-C matching status on the rituximab-mediated ADCC enhancement by HLA-C-uncoupled KIR might depend on the target cells.

Recently, it has become clear that active engagement of inhibitory receptors on NK cells by self-MHC class I molecules is the key event that determines whether an NK cell will be functionally capable of mediating "missing-self" recognition (education). Several models have been proposed to describe various aspects of NK cell education by MHC class I molecules (Hoglund and Brodin). In the "licensing model," NK cells acquire functional competence after ligation of inhibitory receptors by self-MHC class I molecules (Kim et al., 2005). According to this model, NK cells that express KIRs which does not have corresponding self-HLA will not be licensed to response the target cells which does not have a KIR-corresponding HLA (hyporesponsive).

In our system, KIR2DL1-positive (C2-specific) NK cells from donors D, E and F, are considered to be "unlicensed" because these donors lack C2 group HLA-C which corresponds to KIR2DL1 (Table 1). These NK cells are expected to be hyporesponsive to the target cells that express no C2 group of HLA-C, such as 721.221LCL HLA-C*0304 (C1) and 103-LCL (C1/-) cells. According to this, we reanalyzed the data used for Figures 4a and c, merging the results from the donors into licensed or unlicensed categories. As shown in Figure 4e, however, we found no enhancement of natural killing (IgG) or rituximab-mediated ADCC with or without license.

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ADCC Enhancement by HLA-C Mismatch Does Not Occur When Whole NK Cells Were Used

Next, rituximab-mediated ADCC was measured using 103-LCL cells and whole NK cells prepared from four donors (donor A, B, D and F), because rituximab-mediated ADCC enhancement by HLA-C-uncoupled KIR was influenced by HLA-C-matching status with this target.

When whole NK cells were used as an effector, the ADCC levels were highly variable among individuals, irrespectively of whether HLA-C mismatch existed between NK cell donors and target cells, and significant differences were not observed between NK cells prepared from HLA-C-matched and HLA-C-mismatched donors (Fig. 5).

These results consequently indicate that ADCC enhancement is not expected if choosing HLA-C-mismatched donors, as far as whole NK cells are used as a source of allogeneic NK cell infusion in clinical settings.

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DISCUSSION

In our analysis, ADCC enhancement by HLA-C-uncoupled KIR was observed in 721.221LCL transfectants, which may support our hypothesis that rituximab-mediated ADCC is not inhibited by KIRs when the target cells do not express the matched HLA-C ligands (Figs. 4a and b). This finding was also observed in 103-LCL cell as a target when HLA-C mismatch exists between NK cell donors and target cells (Fig. 4c donor A, B and C). Furthermore, when HLA-C mismatch does not exist between NK cell donors and target cells, ADCC enhancement by HLA-C-uncoupled KIR was not seen with 103-LCL (Figs. 4c donor D, E and F), as we expected.

This result raises a suggestion that subjects carrying HLA-C mismatch are better as a donor of allogeneic NK cells. However, the ADCC levels were highly variable among individuals when whole NK cells were used (Fig. 5), and we could not find any evidence that donors with HLA-C mismatch have an advantage. FcγRIII is known to have polymorphism with either a phenylalanine or a valine (V) at amino acid position 158. FcγRIII-158V has higher affinity for human IgG1(Koene et al., 1997), which results in increased ADCC mediated by rituximab (Hatjiharissi et al., 2007). Polymorphisms like this in NK cell donors might have resulted in the variation of ADCC among NK cell donors.

In conclusion, we demonstrated the enhancement of ADCC by HLA class I knockdown of the target cells or by purified NK cells with HLA-C-uncoupled KIR. When whole NK cells were used, however, variation of rituximab-mediated ADCC was substantial among individuals, and HLA-C mismatch between NK cell donors and target cells did not provide significant impact on the level of ADCC. Thus, donors with HLA-C mismatch may not necessarily have an

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advantage when immunotherapy using allogeneic NK cells is considered in combination with rituximab for the treatment of lymphoma.

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Declaration of Interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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Notch2 and Immune Function

Mamiko Sakata-Yanagimoto and Shigeru Chiba

Abstract Notch2 is expressed in many cell types of most lineages in the hematolymphoid compartment and has specific roles in differentiation and function of various immune cells. Notch2 is required for development of splenic marginal zone B cells and regulates differentiation of dendritic cells (DCs) in the spleen. Notch2 appears to play some specific roles in the intestinal immunity, given that the fate of mast cells and a subset of DCs is regulated by Notch2 in the intestine. Notch2 also has important roles in helper T cell divergence from na CD4 T cells and activation of cytotoxic T cells. Moreover, recent genetic evidence suggests that both gain-and loss-of-function abnormalities of Notch2 cause transformation of immune cells. Inactivating mutations are found in Notch2 signaling pathways in chronic myelomonocytic leukemia, while activating mutations are found in mature B cell lymphomas, which reflects the role of Notch2 in the developmental process of these cells.

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7.1 Notch2 Mutations in B Cell Lymphomas 157

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1 Introduction

The expression pattern of Notch1, Notch2, Notch3, and Notch4 varies from one cell type to another. Notch2 plays specific roles in the immune compartment independently of and cooperatively with Notch1 and Notch3. In this chapter, we mostly focus on evidence based on mouse genetic studies regarding Notch2 functions in immune cells. In the last part, we discuss the involvement of Notch2 in neoplastic transformation in conjunction with human hematologicmalignancies.

2 Notch2 Signaling in Marginal Zone B Cell Development

Mature splenic B cells are mainly divided into 2 types of B cells, follicular B (FOB) cells and marginal zone B (MZBs) cells (Martin and Kearney 2002). FOB cells are one of the main effectors of acquired immunity, able to respond to a large variety of antigens, while MZB cells can only elicit an immune response to a limited number of antigens. Immature B cells, developing from hematopoietic stem cells (HSCs) in the bone marrow, migrate to the spleen, and differentiate first into T1 (type1) transitional B cells (characterized by: IgM^{hi} IgD^{lo} $CD21^{lo}$), before differentiating into T2 (type2) transitional B cells (IgM^{hi} IgD^{hi} $CD21^{int}$ $CD23^{hi}$) (Loder et al. 1999). These progenitors further differentiate into the two types of B cells, MZB cells (IgM^{hi} IgD^{lo} $CD21^{hi}$ $CD23^{lo}$) and FOB cells (IgM^{lo} IgD^{hi} $CD21^{int}$ $CD23^{hi}$) (Martin and Kearney 2002). Conditional inactivation of *Notch2* in the B cell lineage results in defective MZB cell development, while FOB cell development is unaffected (Saito et al. 2003), which is basically consistent with the phenotype of *RBP-J* conditional knockout mice (Tanigaki et al. 2002). The cleaved Notch-RBP-J activator complex contains at least one out of three family members of the mastermind-like proteins (MAML1-3). Mastermind-like1 (MAML1) plays an essential role in MZB cell development, which is why MZB cells are lacking in *MAML1*-null mice (Wu et al. 2007; Oyama et al. 2007). Among Notch ligands, Delta-like 1 (Dll1) is responsible for MZB cell development, based on the fact that *Dll1* deletion using the Mx-Cre *loxP* system leads to loss of MZB cells (Hozumi et al. 2004; Sheng et al. 2008). Several lines of evidence indicate that loss of *Dll1* expression on nonhematopoietic cells causes MZB cell defects (Hozumi et al. 2004; Sheng et al. 2008; Tan et al. 2009). However, the exact cell types through which Dll1-induced Notch2 signaling triggers MZB development remains to be elucidated. The essential role of Notch signaling in MZB cell development is further proven by a number of other gene-targeted mice in which

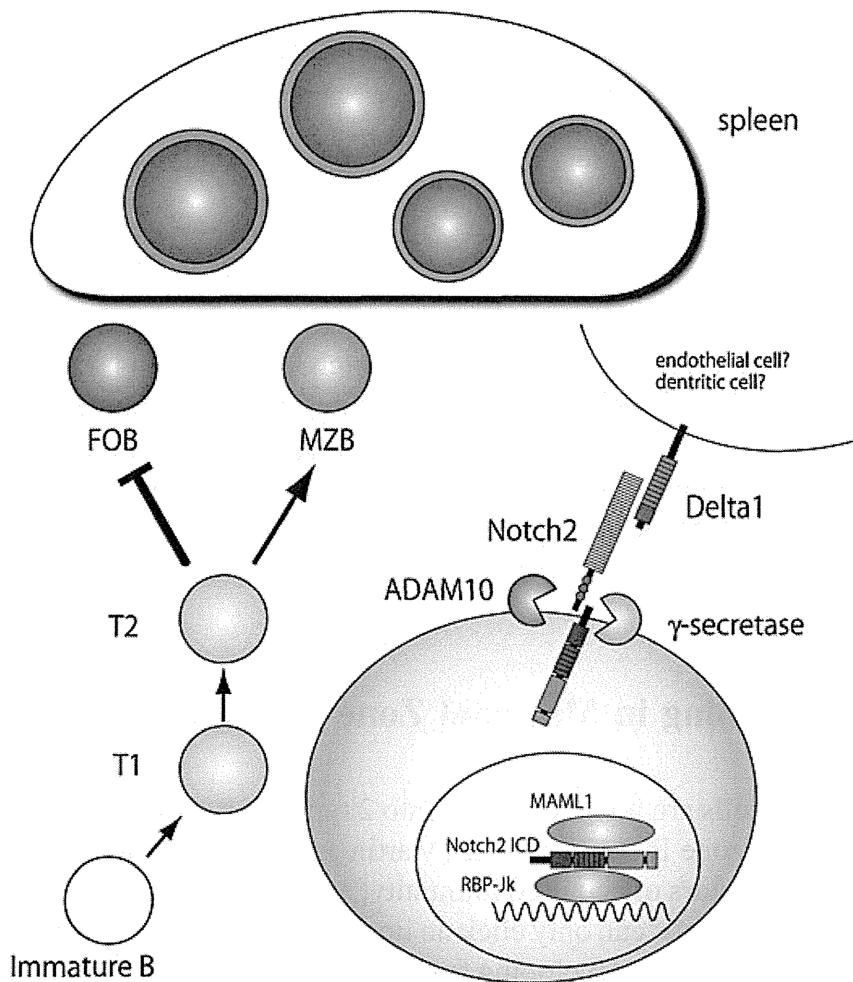

Fig. 1 Notch2 regulates marginal zone B cell development. Dll1 engagement of the Notch2 receptor expressed on splenic immature B cells initiates the Notch2 signaling cascade through ADAM10-mediated cleavage. This results in the formation of Notch-RBP-J-MAML1 proteins, which regulates gene expression and skews the differentiation program toward MZB cells rather than FOB cells

genes of the Notch signaling cascade were inactivated. Mind bomb (Mib), an E3 ubiquitin ligase, activates Notch signaling through endocytosis of Notch ligands. Deletion of *Mib1* in nonhematopoietic cells recapitulates defects in MZBs, whereas deletion in hematopoietic cells resulted in MZB levels that were comparable to control mice (Song et al. 2008).

Deletion of *ADAM10*, which encodes a matrix metalloprotease that processes the extracellular domain of the Notch receptor after ligand binding, also results in defects in MZB cells (Gibb et al. 2010). In contrast, deletion of *Msx2-interacting nuclear target protein* (*MINT*), which is a repressor of RBP-J mediated transcriptional activity and thus considered to be a negative regulator for Notch signaling, showed decreased numbers of FOB cells and increased numbers of MZB cells (Kuroda et al. 2003). Notch receptors can be modified by fringe glycosyl transferases. Lunatic and manic fringe were shown to cooperatively enhance Dll1-Notch2 interaction, and thereby induce MZB development (Tan et al. 2009).

Taken together, these data suggest that Dll1 engagement of the Notch2 receptor expressed on splenic immature B cells initiates the Notch2 signaling cascade through ADAM10-mediated cleavage. This results in the formation of a multi-protein complex including Notch-RBP-J-MAML1 proteins, which regulates gene expression and thereby skews the differentiation program toward MZB cells rather than FOB cells (Fig. 1).

3 Notch2 Signaling in Peripheral T Cell Differentiation and Activation

Signaling through Notch1 has been proven to be among the most important systems for immature T cell differentiation in the thymus (Radtke et al. 1999). During T cell differentiation, Notch signaling is also an essential component for functional maturation and activation of peripheral T cells, in which Notch2 appears to be among the main players (Maekawa et al. 2008). Notch2 expression is increased along with activation of CD8+ cytotoxic T lymphocytes (CTL) (Maekawa et al. 2008). CTLs of both *Notch2* and *RBP-J* conditional knockout mice show an impaired activation potential in vitro as well as in vivo (Maekawa et al. 2008). Cleaved Notch2 (N2IC) directly interacts with CREB and p300 and binds to the promoter of the granzyme B gene, an effector molecule of CTL (Maekawa et al. 2008). Conditional inactivation of *Notch2* in CD8+ T cells results in a decreased antitumor response (Sugimoto et al. 2010). Notch1 appears to be dispensable for an efficient CTL response given the fact that deletion of *Notch1* in CD8+ T cells shows an antitumor response comparable to control mice (Sugimoto et al. 2010). However, this view was recently challenged by a report showing that Notch1 also directly controls main players of CTL, including Eomes, perforin, and granzyme B (Cho et al. 2009).

Notch2/Notch1 double deficient animals reveal impaired differentiation of na CD4+ T cells toward helper T2 (Th2) cells. Notch was shown to directly regulate the transcription of the transcription factor GATA3, and the cytokine interleukin-4 (IL-4) (Amsen et al. 2004, 2007; Fang et al. 2007), both of which are important mediators of Th2 differentiation. *RBP-J* deficient animals recapitulate the phenotype observed in *Notch2/Notch1* deficient animals indicating that this process is mediated through canonical Notch signaling (Amsen et al. 2004). The role of Notch signaling in Th1 differentiation is less clear. Several reports demonstrated that Th1 differentiation is augmented by Notch signaling (Maekawa et al. 2003; Sun et al. 2008); however, a Th1 response is maintained in *Notch2/Notch1* double deficient, and *RBP-J* deficient animals (Amsen et al. 2004), as well as in mice expressing a dominant negative *MAML1* (Tu et al. 2005), questioning the importance of Notch signaling in Th1 differentiation.

With certainty, it can thus be summarized that Notch2 signaling induces cytotoxic T cell differentiation and activation, and that Notch1 and Notch2 concordantly induce Th2 cell differentiation.

4 Notch2 Signaling in Mast Cells

Mast cells arise from HSCs in the bone marrow, migrate to peripheral tissues as immature progenitors, where they subsequently differentiate into mature mast cells (Galli et al. 2005). However, the detailed process of their development is still disputed. Mast cells can be generated in vitro by culturing mouse bone marrow cells with a defined cocktail of cytokines. This in vitro system allows to partially mimic the physiologic development of mast cells. Notch2 signaling instructs myeloid progenitors to adopt a mast cell fate as opposed to differentiate into neutrophils or macrophages, through the coordinated regulation of *Hes1* and *GATA3* (Sakata-Yanagimoto et al. 2008). Mast cells are divided into two subtypes; mucosal and connective tissue type mast cells (Gurish and Boyce 2006; Miller and Pemberton 2002). Each subtype features specific mast-cell proteases (mMCP) (Miller and Pemberton 2002). Notch2 signaling skews cultured mast cells toward the mucosal type rather than connective tissue type (M.S.-Y. and S.C., unpublished data). The *Strongyloides venezuelensis* (*SV*) infection model is useful for analyzing mast cell-mediated mucosal immunity (Maruyama et al. 2000). This nematode evokes intraepithelial mast cell hyperplasia in the small intestine (Maruyama et al. 2000). *Notch2*-null mice show impaired expulsion of *SV*, possibly because of a delayed mast cell progenitor production in the bone marrow, impaired migration of mast cells from the lamina propria to the intraepithelium of the intestine, and impaired activation of intestinal mast cells (Sakata-Yanagimoto et al. 2011). The number and distribution of connective tissue-type mast cells are normal in *Notch2*-null mice (Sakata-Yanagimoto et al. 2011), suggesting that Notch2 signaling is specifically required for proper migration and activation of intestinal mast cells.

5 Notch2 Signaling in Dendritic Cells

Dentritic cells (DCs) initiate immune responses by presenting antigen to na T cells (Steinman and Idoyaga 2010). DCs arise from common bone marrow progenitors that can give rise to both DCs and macrophages (Steinman and Idoyaga 2010). DCs comprise two subclasses, i.e., the so-called plasmacytoid DCs and classical DCs. Classical DCs residing in the spleen are further classified into two main subsets; CD8+CD11b− DCs which mediate cross-presentation to cytotoxic T cells via MHC class I pathway (Dudziak et al. 2007; den Haan et al. 2000) and CD8−CD11b+ DCs which preferentially present MHC class II restricted antigens to CD4+ helper T cells (Dudziak et al. 2007). CD8−CD11b+ DCs are mainly localized in the marginal zone, adjacent to the Dll1-expressing cells (Caton et al. 2007). CD11b+ DCs in the lamina propria of the intestine contain two distinct subsets; CD11b+CD103+ DCs and CD11b+CD103− DCs. CD11b+CD103+ DCs migrate to mesenteric lymph nodes and are presumed to have antigen presenting potential to helper T cells (Denning et al. 2011; Bogunovic et al. 2009).

DC-specific deletion of either *Notch2* or *RBP-J* impairs the development of CD8−CD11b+ DCs in the spleen (Caton et al. 2007; Lewis et al. 2011). CD8+ DCs are also decreased by deletion of *Notch2* but are not affected by the deletion of *RBP-J* (Caton et al. 2007; Lewis et al. 2011). Splenic CD11b+ DCs are divided into two subsets according to the expression levels of Esam and Cx3cr1 (Lewis et al. 2011). CD11b+Esam^{high}Cx3cr1^{low} but not CD11b+Esam^{low}Cx3cr1^{high} DCs are almost abrogated by deletion of either *Notch2* or *RBP-J* (Lewis et al. 2011). CD11b+Esam^{high}Cx3cr1^{low} cells are required for proper priming of T cells in the spleen, which are reduced in *RBP-J*-null mice (Lewis et al. 2011).

Notch2 selectively controls CD11b+CD103+ DCs in the lamina propria of the intestine as well as those that migrate toward mesenteric lymph nodes, which in turn are important for supporting IL-17 producing CD4+ T cells. CD11b+CD103+ DCs are not affected by the inactivation of *RBP-J* (Lewis et al. 2011).

Taken together, Notch2 regulates tissue-specific subsets of DCs in the spleen and in the intestine. Notch2 function might be partly mediated by a RBP-J independent/noncannonical pathway.

6 Notch2 Signaling in Hematopoietic Stem Cells

Notch signaling plays an essential role in self-renewal of stem cells as well as in the growth and differentiation of diverse progenitors within various organs. In contrast, the role of Notch signaling in self-renewal of HSC has been disputed over the years. Early in vitro gain-of-function experiments, such as introduction of a constitutive active form of Notch (Varnum-Finney et al. 2000; Stier et al. 2002) or the transcription factor Hes1 (Kunisato et al. 2003), and stimulation of HSCs with cell-surface expressed ligands or ligand-immunoglobulin chimeric proteins (Karanu et al. JEM 2000; Ohisi et al. JCI 2002; Suluki et al. Stem cells 2006), indicated that Notch signaling supports self-renewal of HSCs and has a role in HSC expansion. On the contrary, several loss-of-function experiments suggest that Notch signaling is dispensable for maintenance of HSCs. HSCs lacking *RBP-J* and those expressing dominant negative *MAML1*, a potent inhibitor of the Notch transcriptional complex, achieve long-term reconstitution comparable to wild-type HSCs, when transplanted into irradiated mice (Maillard et al. 2008). The reconstitution potential of HSCs null for both *Notch1* and *Jagged1* was shown to be comparable to that of wild-type HSCs (Mancini et al. 2005).

However, recently, such negative findings were partially challenged. At a very early time point after treatment with 5-fluorouracil, the number of multipotent progenitors (MPPs) was decreased in *Notch2*-null mice, compared to that in control mice (Varnum-Finney et al. 2011). Similarly, shortly after transplantation, both MPPs and long-term HSCs were decreased in *Notch2*-null BM transplanted mice (Varnum-Finney et al. 2011).

In summary, these data suggest that although Notch signaling is dispensable for homeostasis of HSCs, in challenge and stress situations signaling through Notch2 seems to play a role in the process of HSCs expansion.

7 Notch2 Signaling in Transformation of Blood Cells

7.1 Notch2 Mutations in B Cell Lymphomas

Notch1 is among the most important molecules for physiologic development of T cells (Radtke et al. 1999), and Notch2 is indispensable for MZB cell development (Saito et al. 2003) as described above. Discovery of hyperactivation of Notch1 and Notch2 through gain-of-function mutations in immature T cell neoplasms (Weng et al. 2004) (T cell acute lymphoblastic leukemia or T-ALL in humans) and in subtypes of mature B cell neoplasms (Lee et al. 2009; Troen et al. 2008), respectively, appears to echo the physiologic roles of these molecules in specific lineages and differentiation stages. Those mutations are concentrated in the extracellular heterodimerization (HD) domain and the intracellular proline-, glutamic acid-, serine-, and threonine-rich (PEST) domain of *Notch1* in T-ALL (Weng et al. 2004), and only in the PEST domain of *Notch2* in mature B cell lymphomas (Lee et al. 2009; Troen et al. 2008). The distribution of mutations suggests that hyperactivation of Notch2 signaling in B cell lymphomas still requires binding of the ligand, whereas mutations within the HD domain of *Notch1* in T-ALL results in ligand independent activation of Notch1 signaling. In contrast to the fact that *Notch1* mutations are found in approximately 50 % of T-ALL cases (Weng et al. 2004), *Notch2* mutations were identified in only five out of 63 cases (8 %) of diffuse B-cell lymphoma (Lee et al. 2009) and in two out of 41 cases (5 %) of MZB cell lymphoma (Troen et al. 2008).

The relationship between B cell development and gain-of-function mutations in Notch2 is not as clear as in the context of T cell development and Notch1 mutations. Genetic evidence described above strongly suggests an oncogenic role of deregulated Notch2 in B lineage transformations. On the contrary, there has been a series of reports describing the tumor suppressive function of Notch signaling in B lineage cells, particularly in B-cell lymphoblastic leukemia (B-ALL) (Zweidler-McKay et al. 2005; Kannan et al. 2011), although loss-of-function mutations have not been found in the Notch2 signaling pathway. Integrating these pieces of information, it seems likely that Notch2 signaling can context dependently promote or suppress growth of B lineage cells. Another complexity was recently added by the identification of Notch1 mutations through the genome-wide screening of patient samples suffering from chronic lymphocytic leukemia (Puente et al. 2011), a type of intermediately mature B cell neoplasm and mantle cell lymphoma (Kridel et al. 2012), another type of mature B neoplasms.

7.2 Notch2 Signaling in Myeloid Neoplasms

Recently, Notch signaling was proven to function as a tumor-suppressor in chronic myelomonocytic leukemia (CMML) (Klinakis et al. 2011); several components of the Notch pathway, including *Nicastrin* (*NCSTN*), *APH1A*, *MAML1*, and *Notch2*

Table 1 Role of Notch signaling as tumor activator or tumor suppressor in hematopoietic leukemia/lymphoma

	Gain-of-function			**Loss-of-function**	
	T	B	M	B	M
Notch1	T-ALL ATL	CLL MCL DLBCL	AML	(B-ALL)[a]	
Notch2		MZB lymphoma DLBCL			CMML

T-ALL, T-cell acute lymphocytic leukemia; ATL, adult T-cell leukemia/lymphoma; CLL, chronic lymphocytic leukemia; MCL, mantle cell lymphoma; DLBCL, diffuse large B-cell lymphoma; MZB lymphoma, marginal zone B-cell lymphoma; AML, acute myeloid leukemia; B-ALL, B-cell acute lymphocytic leukemia; CMML, chronic myelomonocytic leukemia

[a] Loss-of-function mutations in Notch signal components have not been found in B-ALL

itself were found to be mutated and defective in CMML patients. This conclusion is also supported by the phenotype of mice lacking *NCSTN*, a component of γ-secretase, as well as that of *Notch1-*, *Notch2-*, and *Notch3-* triple null mice (Klinakis et al. 2011). These animals show enhanced granulocyte-monocyte progenitor potential and develop a fatal CMML-like disease (Klinakis et al. 2011). On the contrary, activating mutations of Notch1 were found in acute myeloid leukemia, a precursor myeloid neoplasms, though the frequency is less than 1 % (Wouters et al. 2007; Fu et al. 2006). Thus, as is the case of B cell malignancies, Notch signaling can function as either tumor promoter or suppressor within myeloid neoplasms.

These oncogenic and tumor suppressive functions of Notch1 and Notch2 signaling in T cell, B cell, and myeloid lineages have been summarized in Table 1. Knowledge about this area will expand rapidly in the very near future using current sequencing technology.

8 Conclusion

Signaling through Notch2 has an essential role in two major cell types present in the marginal zone of the spleen, splenic MZB cells, and splenic DCs. Notch2 signaling also mediates intestinal immunity by regulating development and localization of intestinal DCs and mast cells, and development of helper T cells and CTLs. Genetic and biologic evidence indicates that abnormal Notch2 signaling is involved in transformation of immune cells, although its functions appear to be bivalent; oncogenic signaling for mature B neoplasms and tumor suppressive signaling for mature myeloid neoplasms. The reason of specificity and non-redundant functions of Notch2 in the immune system may be partly attributed to the differences in expression patterns among Notch family genes, although this issue needs to be elucidated in more detail in future studies.

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