J Clin Oncol. 2010 Feb 20;28(6):955-9. Epub 2010 Jan 19.

NKAML: a pilot study to determine the safety and feasibility of haploidentical natural killer cell transplantation in childhood acute myeloid leukemia.

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PURPOSE To conduct a pilot study to determine the safety, feasibility, and engraftment of haploidentical natural killer (NK) cell infusions after an immunosuppressive regimen in children with acute myeloid leukemia (AML). PATIENTS AND METHODS Ten patients (0.7 to 21 years old) who had completed chemotherapy and were in first complete remission of AML were enrolled on the Pilot Study of Haploidentical Natural Killer Cell Transplantation for Acute Myeloid Leukemia (NKAML) study. They received cyclophosphamide (60 mg/kg on day -7) and fludarabine (25 mg/m(2)/d on days -6 through -2), followed by killer immunoglobulin-like receptor-human leukocyte antigen (KIR-HLA) mismatched NK cells (median, 29 x 10(6)/kg NK cells) and six doses of interleukin-2 (1 million U/m(2)). NK cell chimerism, phenotyping, and functional assays were performed on days 2, 7, 14, 21, and 28 after transplantation. Results All patients had transient engraftment for a median of 10 days (range, 2 to 189 days) and a significant expansion of KIR-mismatched NK cells (median, 5,800/mL of blood on day 14). Nonhematologic toxicity was limited, with no graft-versus-host disease. Median length of hospitalization was 2 days. With a median follow-up time of 964 days (range, 569 to 1,162 days), all patients remain in remission. The 2-year event-free survival estimate was 100% (95% CI, 63.1% to 100%). CONCLUSION Low-dose immunosuppression followed by donor-recipient inhibitory KIR-HLA mismatched NK cells is well tolerated by patients and results in successful engraftment. We propose to further investigate the efficacy of KIR-mismatched NK cells in a phase II trial as consolidation therapy to decrease relapse without increasing mortality in children with AML.

Science. 2002 Mar 15;295(5562):2094-7.

Influence of SHIP on the NK repertoire and allogeneic bone marrow transplantation.

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Comment in:
Science. 2002 Mar 15;295(5562):2029-31.

Natural killer cell (NK) receptors for major histocompatibility complex (MHC) class I influence engraftment and graft-versus-tumor effects after allogeneic bone marrow transplantation. We find that SH2-containing inositol phosphatase (SHIP) influences the repertoire of NK receptors. In adult SHIP-/- mice, the NK compartment is dominated by cells that express two inhibitory receptors capable of binding either self or allogeneic MHC ligands. This promiscuous repertoire has significant functional consequences, because SHIP-/- mice fail to reject fully mismatched allogeneic marrow grafts and show enhanced survival after such transplants. Thus, SHIP plays an important role in two processes that limit the success of allogeneic marrow transplantation: graft rejection and graft-versus-host disease.

Science. 2002 Mar 15;295(5562):2097-100.

Effectiveness of donor natural killer cell alloreactivity in mismatched hematopoietic transplants.

Ruggeri L, Capanni M, Urbani E, Perruccio K, Shlomchik WD, Tosti A, Posati S, Rogaia D, Frassoni F, Aversa F, Martelli MF, Velardi A.
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Comment in:
Science. 2002 Mar 15;295(5562):2029-31.

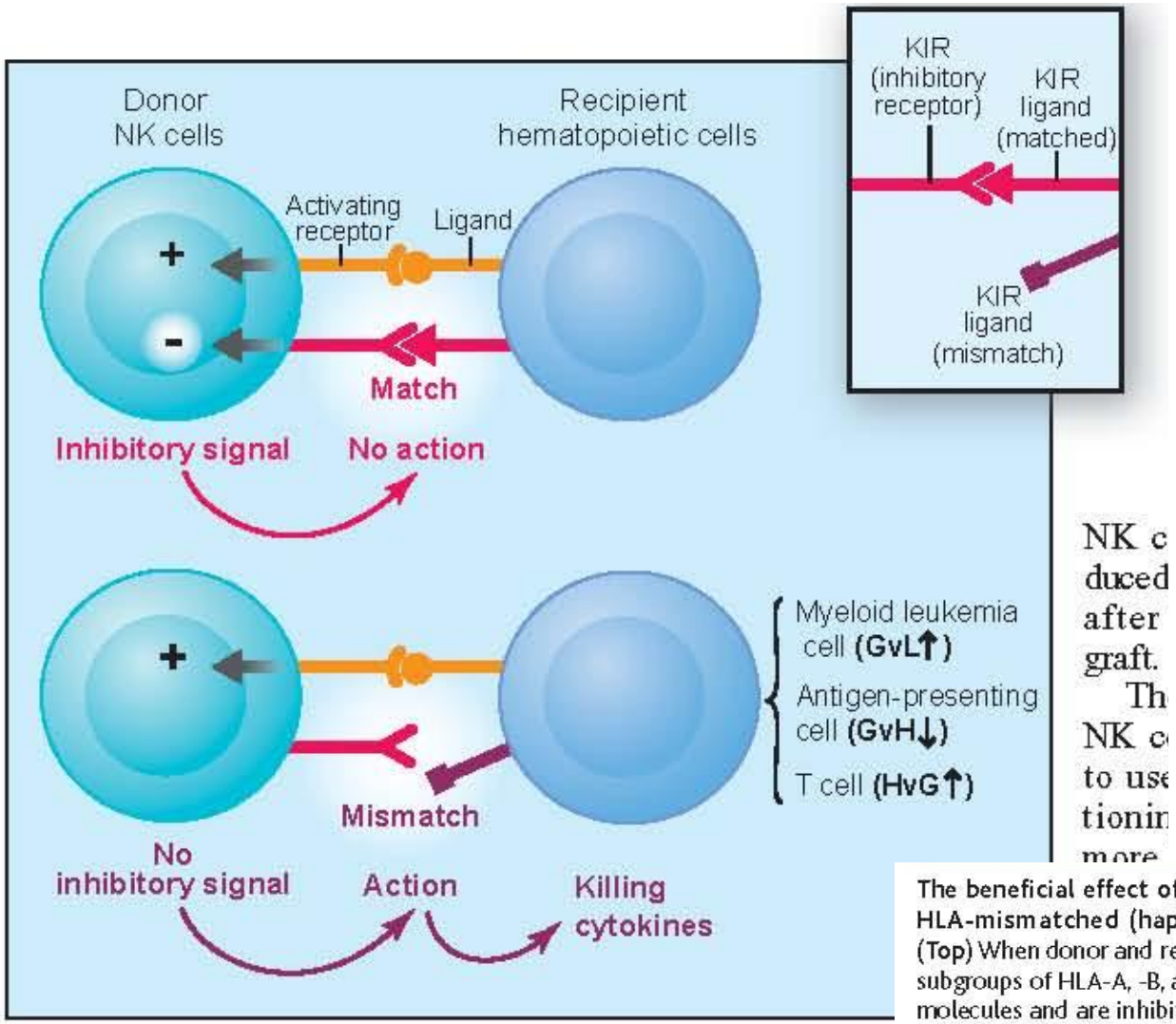
T cells that accompany allogeneic hematopoietic grafts for treating leukemia enhance engraftment and mediate the graft-versus-leukemia effect. Unfortunately, alloreactive T cells also cause graft-versus-host disease (GVHD). T cell depletion prevents GVHD but increases the risk of graft rejection and leukemic relapse. In human transplants, we show that donor-versus-recipient natural killer (NK)-cell alloreactivity could eliminate leukemia relapse and graft rejection and protect patients against GVHD. In mice, the pretransplant infusion of alloreactive NK cells obviated the need for high-intensity conditioning and reduced GVHD. NK cell alloreactivity may thus provide a powerful tool for enhancing the efficacy and safety of allogeneic hematopoietic transplantation.

Science. 2002 Mar 15;295(5562):2029-31.

Immunology. A perfect mismatch.

Kärre K.
Microbiology and Tumor Biology Center, Karolinska Institute, Stockholm, Sweden.

Comment on:
Science. 2002 Mar 15;295(5562):2097-100.
Science. 2002 Mar 15;295(5562):2094-7.



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The beneficial effect of natural killer (NK) cells on the outcome of partly HLA-mismatched (haploidentical) hematopoietic cell transplantation. (**Top**) When donor and recipient tissues are matched for KIR ligands—which are subgroups of HLA-A, -B, and -C alleles—NK cells can sense the appropriate HLA molecules and are inhibited from further action. (**Bottom**) In the case of a KIR ligand mismatch, the KIRs of NK cells do not engage ligand and the action initially elicited by activating receptors proceeds. NK cell attack of leukemia cells may explain the graft-versus-leukemia (GvL) effect, whereas NK cell attack of host antigen-presenting cells may explain why there is less of a graft-versus-host (GvH) reaction against nonhematopoietic tissues of the host. Attack of host T cells by NK cells may explain how NK cells prevent rejection of the graft by the host and suggests that the patient may benefit from receiving donor NK cells as part of a pretransplant conditioning regimen. NK cells express several types of activating and inhibitory receptors, and so there may be other processes that affect GvL, GvH, and HvG. T cells can also affect these reactions.

Blood. 2004 May 15;103(10):3655-61. Epub 2004 Jan 29.

Allogeneic bone marrow transplantation for children with acute myelocytic leukemia in first remission demonstrates a role for graft versus leukemia in the maintenance of disease-free survival.

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In Children's Cancer Group (CCG) study 2891, patients who were recently diagnosed with acute myelocytic leukemia (AML) were assigned randomly to standard- or intensive-timing induction chemotherapy. Patients in first complete remission (CR1) and who had a human leukocyte antigen (HLA)-identical, related donor or a donor disparate at a single class I or II locus were nonrandomly assigned to receive a bone marrow transplant (BMT) by using oral busulfan (16 mg/kg) and cyclophosphamide (200 mg/kg). Methotrexate only was given for graft-versus-host disease (GVHD) prophylaxis. One hundred fifty patients received transplants. Grade 3 or 4 acute GVHD occurred in 9% of patients. Patients younger than 10 years had a lower incidence of grade 3 or 4 GVHD (4.6%) compared with patients 10 years or older (17.4%) (P =.044). Disease-free survival (DFS) at 6 years was 67% and 42% for recipients of intensive- and standard-timing induction therapies, respectively. Multivariate analysis showed that receiving intensive-timing induction therapy (P =.027) and having no hepatomegaly at diagnosis (P =.009) was associated with favorable DFS, and grades 3 and 4 acute GVHD were associated with inferior DFS. Multivariate analysis showed that grades 1 or 2 GVHD (P =.008) and no hepatomegaly at diagnosis (P =.014) were associated with improved relapse-free survival (RFS). Our results show that children older than 10 years are at higher risk for developing severe GVHD; acute GVHD is associated with favorable RFS.

Blood. 2005 Apr 15;105(8):3051-7. Epub 2005 Jan 4.

Successful adoptive transfer and in vivo expansion of human haploidentical NK cells in patients with cancer.

Miller JS, Soignier Y, Panoskaltsis-Mortari A, McNearney SA, Yun GH, Fautsch SK, McKenna D, Le C, Defor TE, Burns LJ, Orchard PJ, Blazar BR, Wagner JE, Slungaard A, Weisdorf DJ, Okazaki IJ, McGlave PB.

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We previously demonstrated that autologous natural killer (NK)-cell therapy after hematopoietic cell transplantation (HCT) is safe but does not provide an antitumor effect. We hypothesize that this is due to a lack of NK-cell inhibitory receptor mismatching with autologous tumor cells, which may be overcome by allogeneic NK-cell infusions. Here, we test haploidentical, related-donor NK-cell infusions in a nontransplantation setting to determine safety and in vivo NK-cell expansion. Two lower intensity outpatient immune suppressive regimens were tested: (1) low-dose cyclophosphamide and methylprednisolone and (2) fludarabine. A higher intensity inpatient regimen of high-dose cyclophosphamide and fludarabine (Hi-Cy/Flu) was tested in patients with poor-prognosis acute myeloid leukemia (AML). All patients received subcutaneous interleukin 2 (IL-2) after infusions. Patients who received lower intensity regimens showed transient persistence but no in vivo expansion of donor cells. In contrast, infusions after the more intense Hi-Cy/Flu resulted in a marked rise in endogenous IL-15, expansion of donor NK cells, and induction of complete hematologic remission in 5 of 19 poor-prognosis patients with AML. These findings suggest that haploidentical NK cells can persist and expand in vivo and may have a role in the treatment of selected malignancies used alone or as an adjunct to HCT.

Blood. 2007 Jul 1;110(1):433-40. Epub 2007 Mar 19.

Donor natural killer cell allorecognition of missing self in haploidentical hematopoietic transplantation for acute myeloid leukemia: challenging its predictive value.

Ruggeri L, Mancusi A, Capanni M, Urbani E, Carotti A, Aloisi T, Stern M, Pende D, Perruccio K, Burchielli E, Topini F, Bianchi E, Aversa F, Martelli MF, Velardi A.
Division of Hematology and Clinical Immunology, Department of Clinical and Experimental Medicine, University of Perugia, Istituto di Ricovero e Cura a Carattere Scientifico, Foundation on Transplantation Biotechnologies, Perugia, Italy.

We analyzed 112 patients with high-risk acute myeloid leukemia (61 in complete remission [CR]; 51 in relapse), who received human leukocyte-antigen (HLA)-haploidentical transplants from natural killer (NK) alloreactive (n = 51) or non-NK alloreactive donors (n = 61). NK alloreactive donors possessed HLA class I, killer-cell immunoglobulin-like receptor (KIR) ligand(s) which were missing in the recipients, KIR gene(s) for missing self recognition on recipient targets, and alloreactive NK clones against recipient targets. Transplantation from NK-alloreactive donors was associated with a significantly lower relapse rate in patients transplanted in CR (3% versus 47%) (P > .003), better event-free survival in patients transplanted in relapse (34% versus 6%, P = .04) and in remission (67% versus 18%, P = .02), and reduced risk of relapse or death (relative risk versus non-NK-alloreactive donor, 0.48; 95% CI, 0.29-0.78; P > .001). In all patients we tested the "missing ligand" model which pools KIR ligand mismatched transplants and KIR ligand-matched transplants from donors possessing KIR(s) for which neither donor nor recipient have HLA ligand(s). Only transplantation from NK-alloreactive donors is associated with a survival advantage.

Annu Rev Immunol. 2008;26:389-420.

Death by a thousand cuts: granzyme pathways of programmed cell death.

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The granzymes are cell death-inducing enzymes, stored in the cytotoxic granules of cytotoxic T lymphocytes and natural killer cells, that are released during granule exocytosis when a specific virus-infected or transformed target cell is marked for elimination. Recent work suggests that this homologous family of serine esterases can activate at least three distinct pathways of cell death. This redundancy likely evolved to provide protection against pathogens and tumors with diverse strategies for evading cell death. This review discusses what is known about granzyme-mediated pathways of cell death as well as recent studies that implicate granzymes in immune regulation and extracellular proteolytic functions in inflammation.

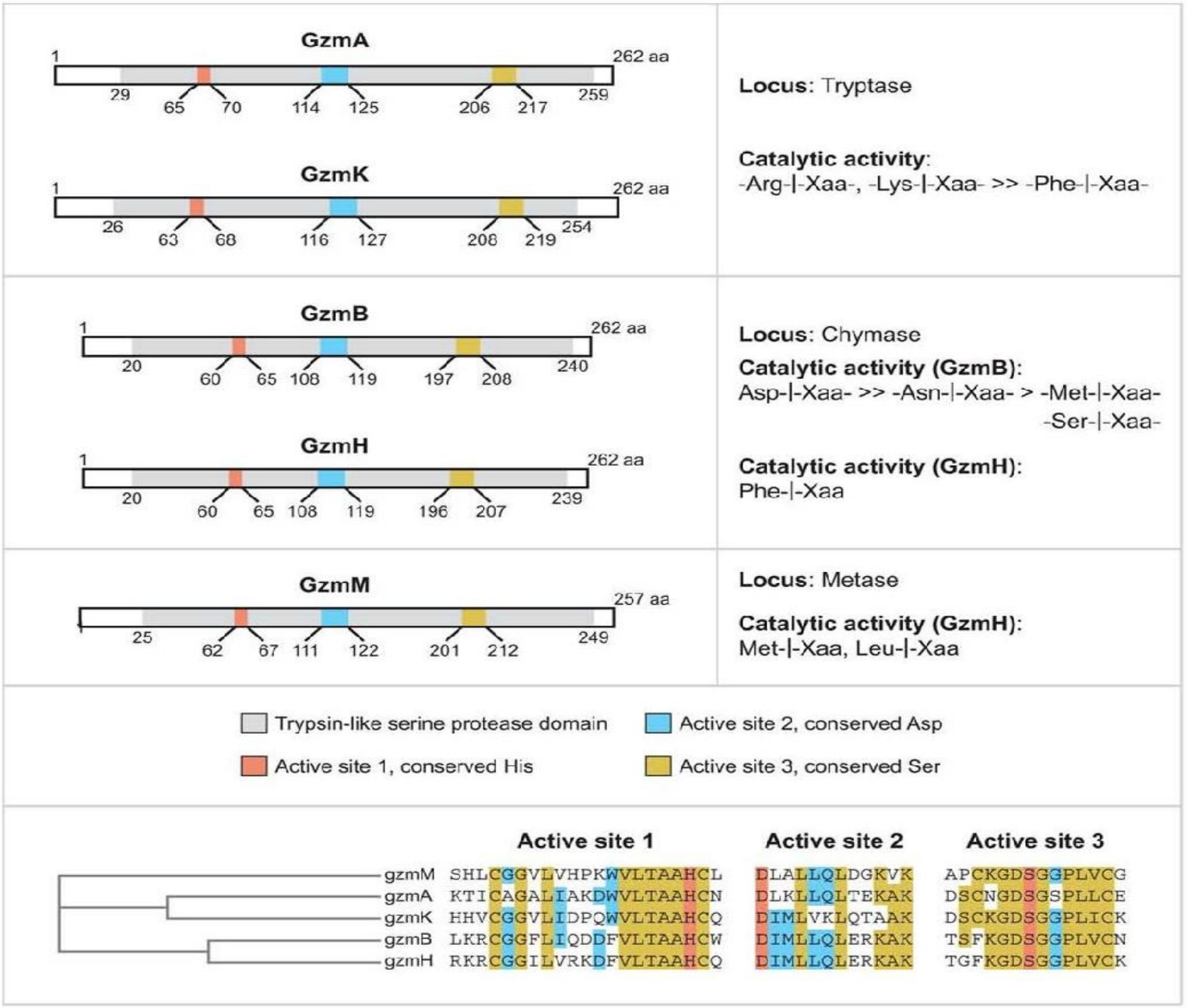

Figure 1.
The family of human granzymes are encoded in three clusters.

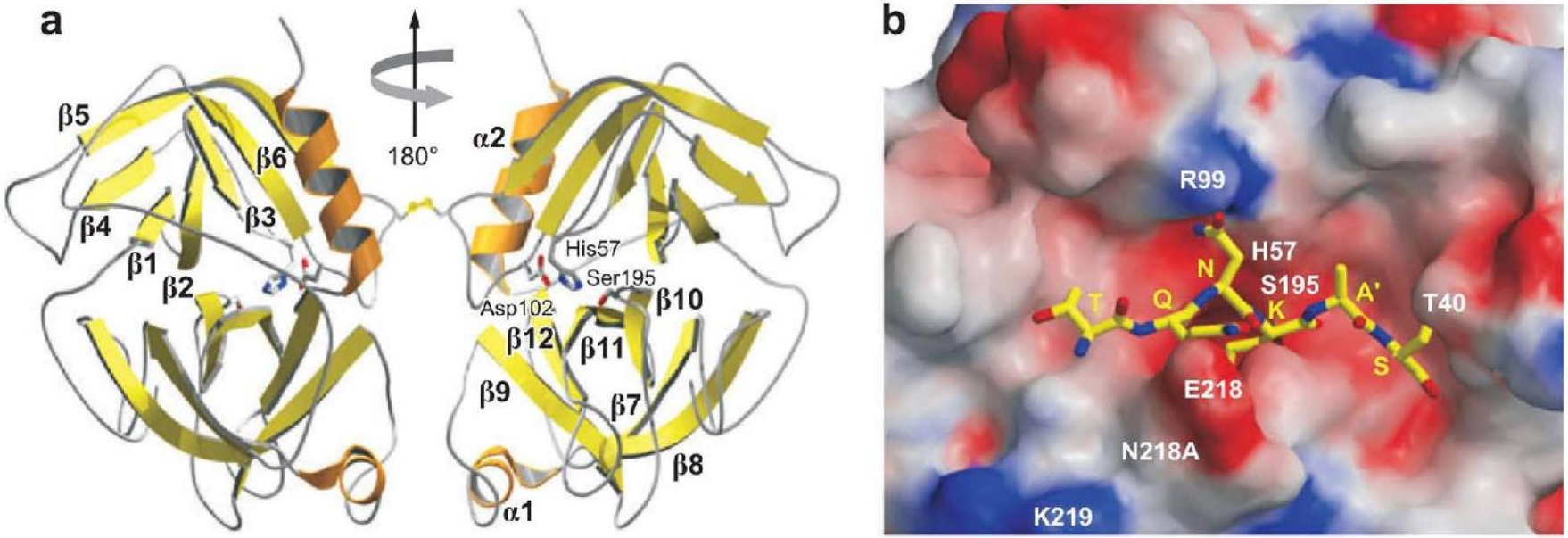

Figure 2.
Crystal structure of the GzmA homodimer. (*a*) GzmA is a disulfide-linked dimer in which the two active sites, indicated on the right (His57-Asp102-Ser195), face in opposite directions. The surface of the molecule contains concentrations of basic amino acids, which may explain the preference for acidic protein substrates through binding outside the active site through an extended exosite. (*b*) The SET protein is an important target of GzmA, whose cleavage triggers its unique pathway of DNA damage. Model of how the SET peptide surrounding the GzmA cleavage site fits into the GzmA active site. [Figures based on the structure obtained by Hink-Shauer and colleagues (54a), reprinted with permission.]

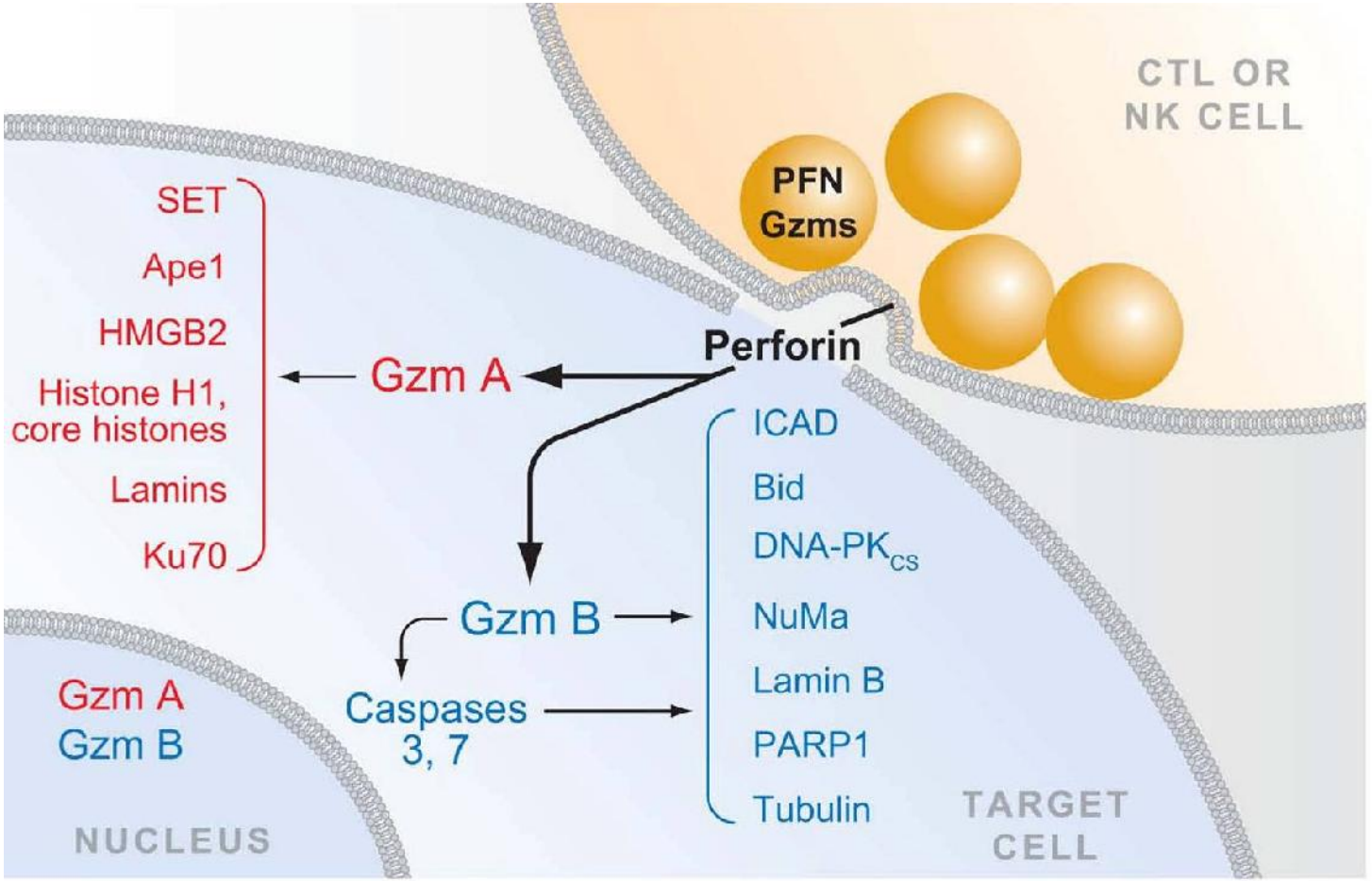

Figure 3.
Granule exocytosis-mediated cell death. When a CTL or NK cell recognizes a target cell, cytolytic granules containing perforin (PFN) and granzymes move to the immune synapse, and the granule membranes fuse with the killer cell plasma membrane, releasing PFN and granzymes into the synapse. PFN facilitates the entry of granzymes into the cytosol of the target cell. The most abundant granzymes are GzmA and GzmB. GzmA activates cell death independently of the caspases, whereas GzmB activates the caspase pathway both directly by cleaving the caspases and indirectly by cleaving key caspase substrates. Some of the key substrates of human GzmA and GzmB are shown. Both GzmA and GzmB traffic to the nucleus by an unknown pathway, where many of the nuclear substrates are cleaved.

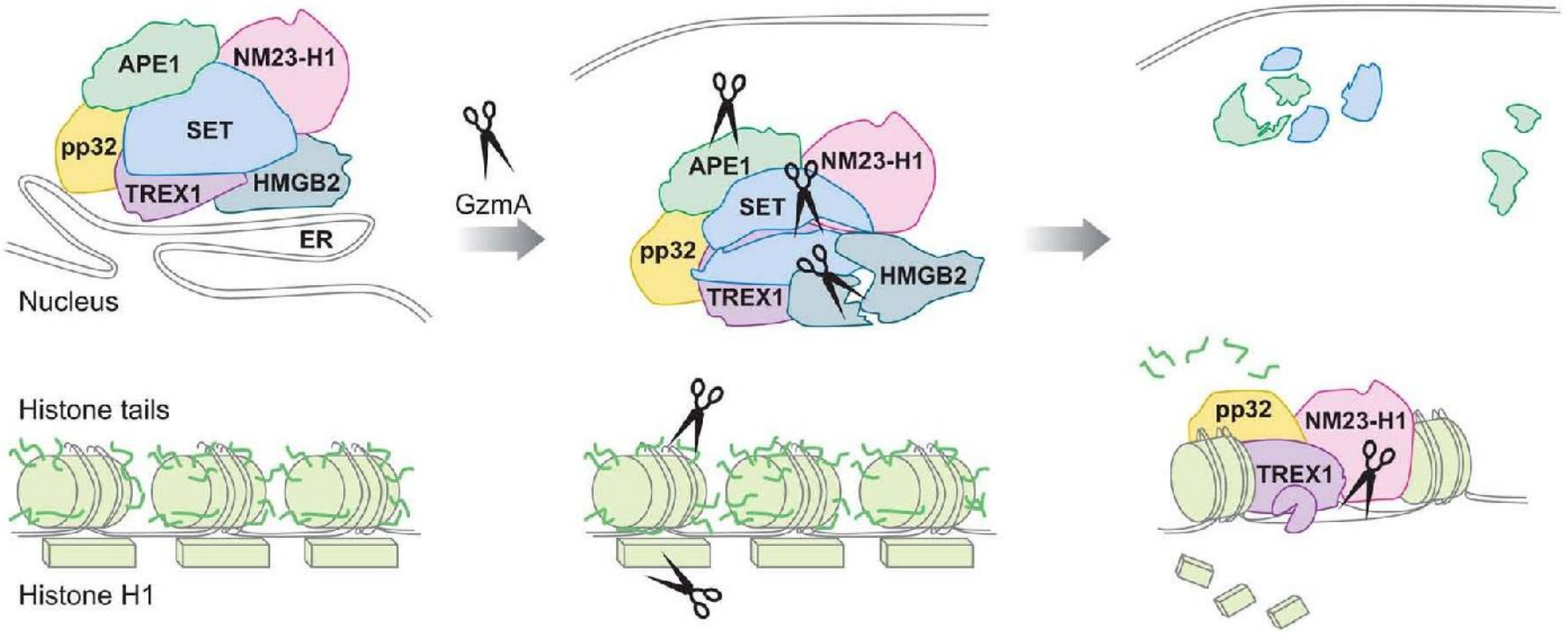

Figure 4.
The GzmA pathway of DNA damage. ROS generated by GzmA in mitochondria drives the ER-associated SET complex into the nucleus. GzmA also enters the nucleus by an unknown pathway. In the nucleus, GzmA cleaves three components of the SET complex (SET, HMGB2, and APE1) to activate two nucleases in the complex to make single-stranded DNA lesions—NM23-H1 makes a nick, which is extended by the exonuclease TREX1. GzmA also degrades the linker histone H1 and removes the tails from the core histones, opening up chromatin and making it more accessible to these nucleases.

Adv Cancer Res. 2008;101:277-348.

The role of NKT cells in tumor immunity.

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NKT cells are a relatively newly recognized member of the immune community, with profound effects on the rest of the immune system despite their small numbers. They are true T cells with a T cell receptor (TCR), but unlike conventional T cells that detect peptide antigens presented by conventional major histocompatibility (MHC) molecules, NKT cells recognize lipid antigens presented by CD1d, a nonclassical MHC molecule. As members of both the innate and adaptive immune systems, they bridge the gap between these, and respond rapidly to set the tone for subsequent immune responses. They fill a unique niche in providing the immune system a cellular arm to recognize lipid antigens. They play both effector and regulatory roles in infectious and autoimmune diseases. Furthermore, subsets of NKT cells can play distinct and sometimes opposing roles. In cancer, type I NKT cells, defined by their invariant TCR using Valpha14Jalpha18 in mice and Valpha24Jalpha18 in humans, are mostly protective, by producing interferon-gamma to activate NK and CD8(+) T cells and by activating dendritic cells to make IL-12. In contrast, type II NKT cells, characterized by more diverse TCRs recognizing lipids presented by CD1d, primarily inhibit tumor immunity. Moreover, type I and type II NKT cells counter-regulate each other, forming a new immunoregulatory axis. Because NKT cells respond rapidly, the balance along this axis can greatly influence other immune responses that follow. Therefore, learning to manipulate the balance along the NKT regulatory axis may be critical to devising successful immunotherapies for cancer.

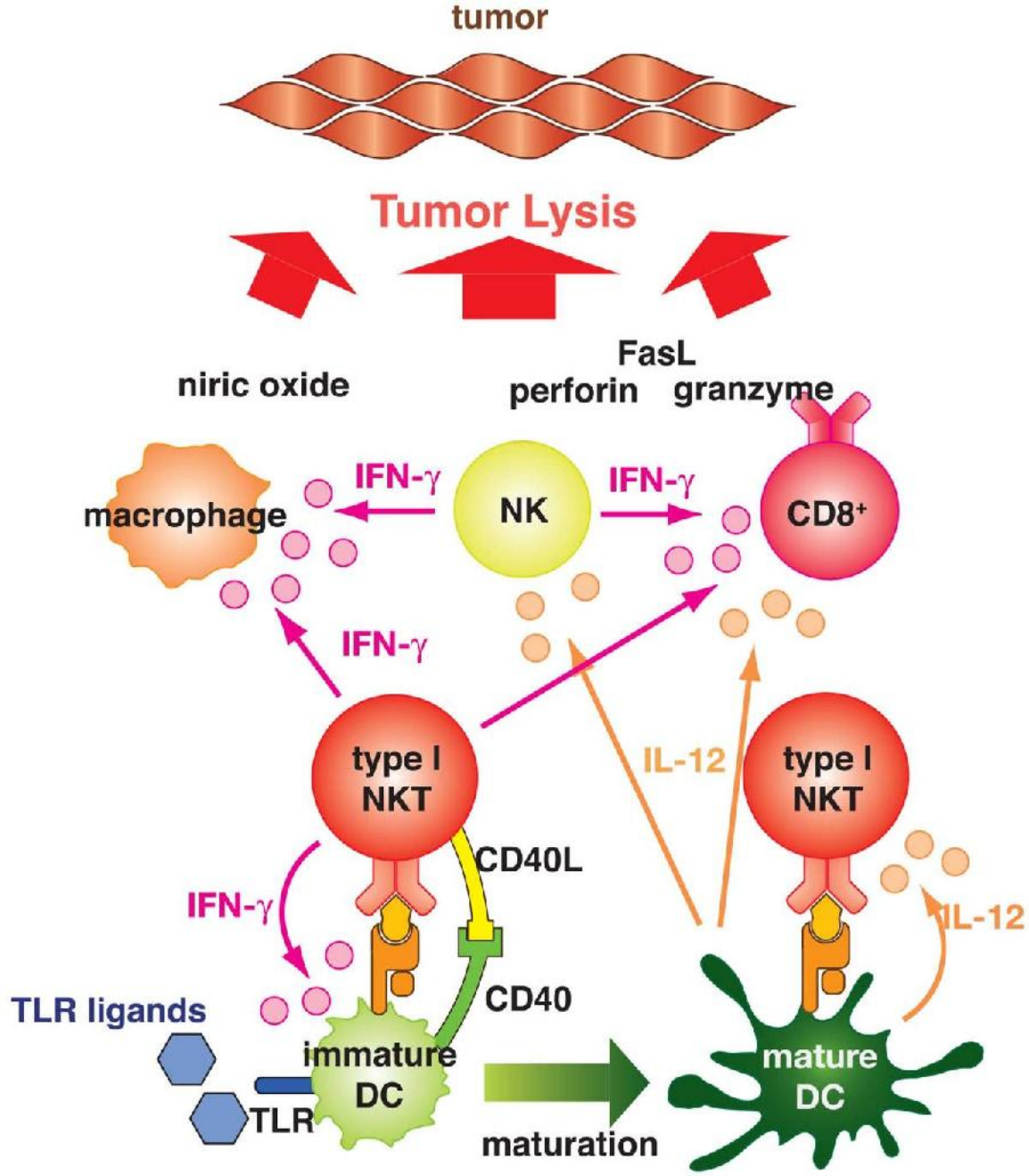

Fig. 1.
Type I NKT cells promote tumor immunity. When type I NKT cells are activated by α-GalCer or endogenous glycolipids (may be tumor
derived) presented by CD1d on immature dendritic cells (DCs), they produce interferon-γ
(IFN-γ). The type I NKT cells may also interact with the immature DCs through CD40-CD40L.
This interaction and IFN-γ induce maturation of the DCs. The mature DCs produce IL-12,
which augments IFN-γ and IL-2 production by type I NKT cells. IFN-γ and IL-2 from the type
I NKT cells and IL-12 from the mature DCs activate NK cells, CD8+ T cells, and macrophages.
Exogenous IL-12 may bypass the process of DC maturation induced by the activated type I
NKT cells. Providing exogenous Toll-like receptor (TLR) ligands may strengthen the cytokine production. Cross-presentation of tumor antigens by antigen presenting cells to CD8+ T cells when activated by the type I NKT cells may enhance induction of tumor antigen-specific
CD8+ T cells. These activated T cells lyse tumor cells by employing multiple effector mechanisms including perforin, granzyme, FasL, and nitric oxide.

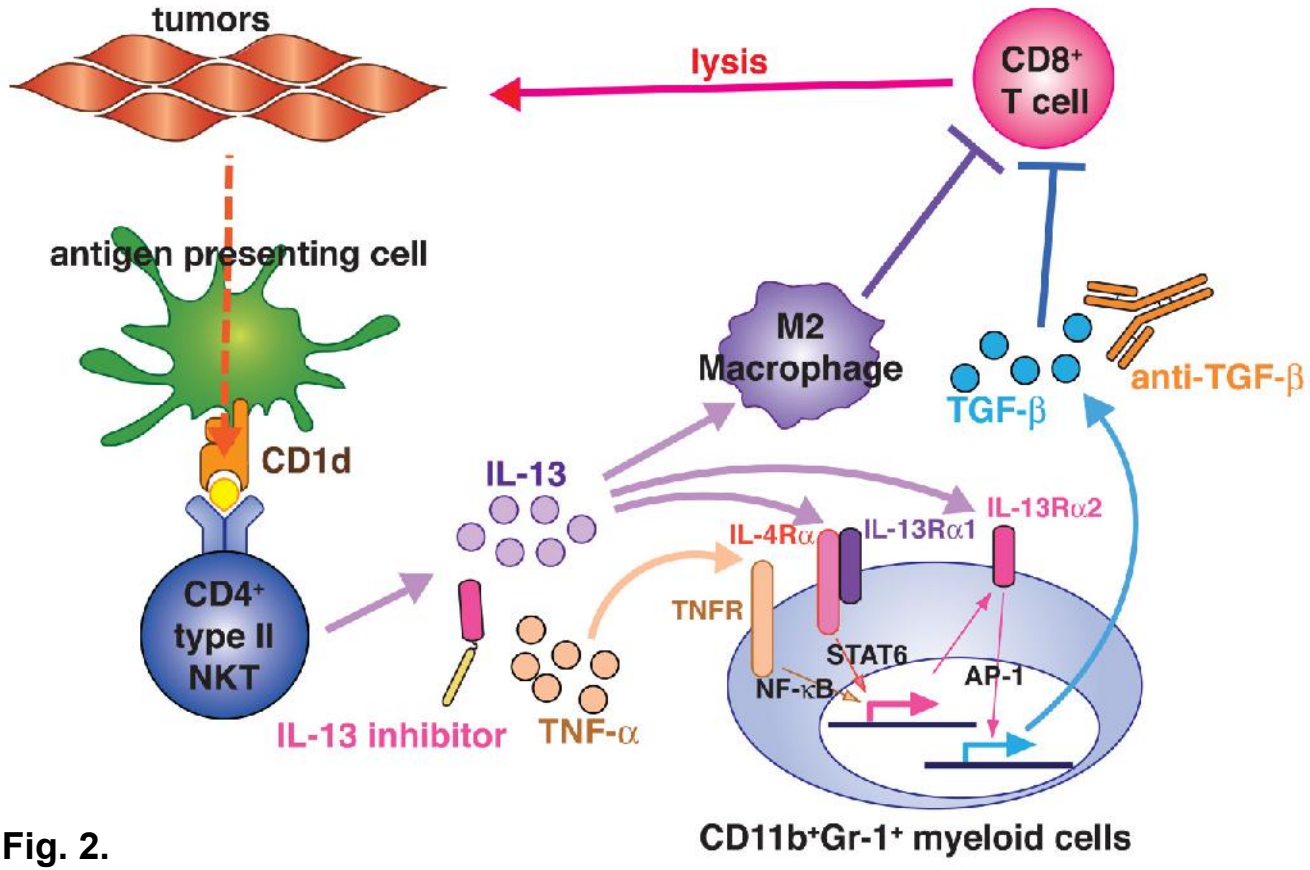

Fig. 2.
Type II NKT cells suppress tumor immunity.
When type II NKT cells (mostly CD4+) are activated by tumor-derived glycolipids presented by CD1d, they produce IL-13. Together with TNF-α in the microenvironment signaling through TNF-receptor (TNFR) and NF-κB, IL-13 signals through a type II IL-4 receptor (IL-4R), a heterodimer of an IL-4Rα and an IL-13Rα1, and STAT6 to induce expression of the IL-13Rα2 on a CD11b+Gr-1+ myeloid cell. The IL-13Rα2 binding to IL-13 transduces a signal through AP-1, which induces expression of TGF-β. TGF-β suppresses activation of tumor specific CD8+ T cells, which mediate regression of tumors. In some tumor settings, IL-13 may induce M2 macrophages that also suppress CD8+ T cells. Blockade of either IL-13 by an IL-13 inhibitor such as soluble IL-13Rα2, or TGF-β with anti-TGF-β antibodies, or TNF-α with a TNF-α antagonist can remove the suppression. Modified from (Terabe, et al., 2003a) with permission.

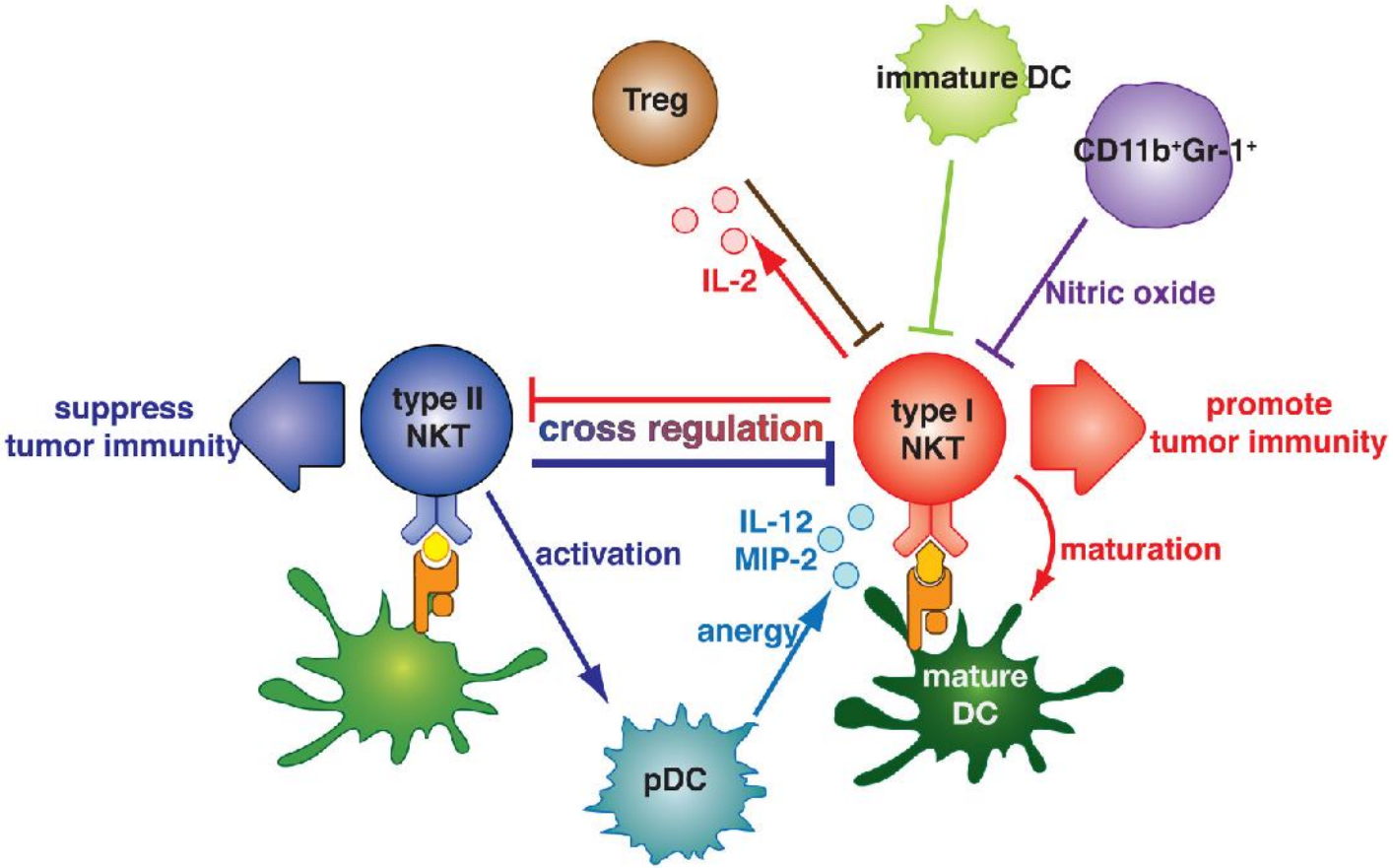

Fig. 3.
Cross-regulation of type I and type II NKT cells—a new immunoregulatory axis.
Type I and type II NKT cells cross-regulate each other. Type II NKT cells suppress tumor immunity when they are activated (by recognizing sulfatide or another lipid presented by CD1d). In some settings, the type II NKT cells suppress type I NKT cells. It is reported that sulfatide activated type II NKT cells activate plasmacytoid DCs (pDC) to produce IL-12 and MIP-2, which recruit and lead to the anergy of type I NKT cells. Activated type I NKT cells induce DC maturation and promote tumor immunity. It is also possible that IL-2 production by activated type I NKT cells supports regulatory T cells, which can suppress type I NKT cells. Immature DCs and CD11b+Gr-1+ myeloid cells may also suppress type I NKT cells in some tumor settings. The cross-regulation between type I and type II NKT cells defines a new immunoregulatory axis like the Th1-Th2 axis. The balance along this axis may in part determine the outcome of tumor immunity. Manipulation of this balance may be critical for the successful immunotherapy of cancer.

J Autoimmun. 2008 May;30(3):172-9. Epub 2008 Jan 31.

Separation of graft-vs.-tumor effects from graft-vs.-host disease in allogeneic hematopoietic cell transplantation.

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Allogeneic hematopoietic cell transplantation (HCT) is an increasingly widely used treatment modality in hematological malignancies. Alloreactivity mediated by donor T cells (and, in some settings, by donor natural killer cells) can produce durable immunologic control or eradication of residual malignancy after allogeneic HCT. However, graft-vs.-tumor (GVT) effects are variably effective and are often accompanied by deleterious alloreactivity against normal host tissue, manifesting as graft-vs.-host disease (GVHD). A major focus of current research in HCT is the separation of beneficial GVT effects from GVHD. Here we review a number of approaches currently under investigation to specifically augment GVT effects, including the identification of minor histocompatibility antigens (mHA), adoptive immunotherapy with tumor-specific or mHA-specific cytotoxic T lymphocytes, vaccination of the donor or recipient to stimulate tumor-specific immunity, and adoptive transfer of natural killer cells. In addition, we review strategies being investigated to specifically suppress GVHD while sparing GVT, including the manipulation and infusion of regulatory T cells, the use of novel pharmacologic and biologic agents, and the use of mesenchymal stem cells. Ultimately, advances in separation of GVT from GVHD will further enhance the potential of allogeneic HCT as a curative treatment for hematological malignancies.

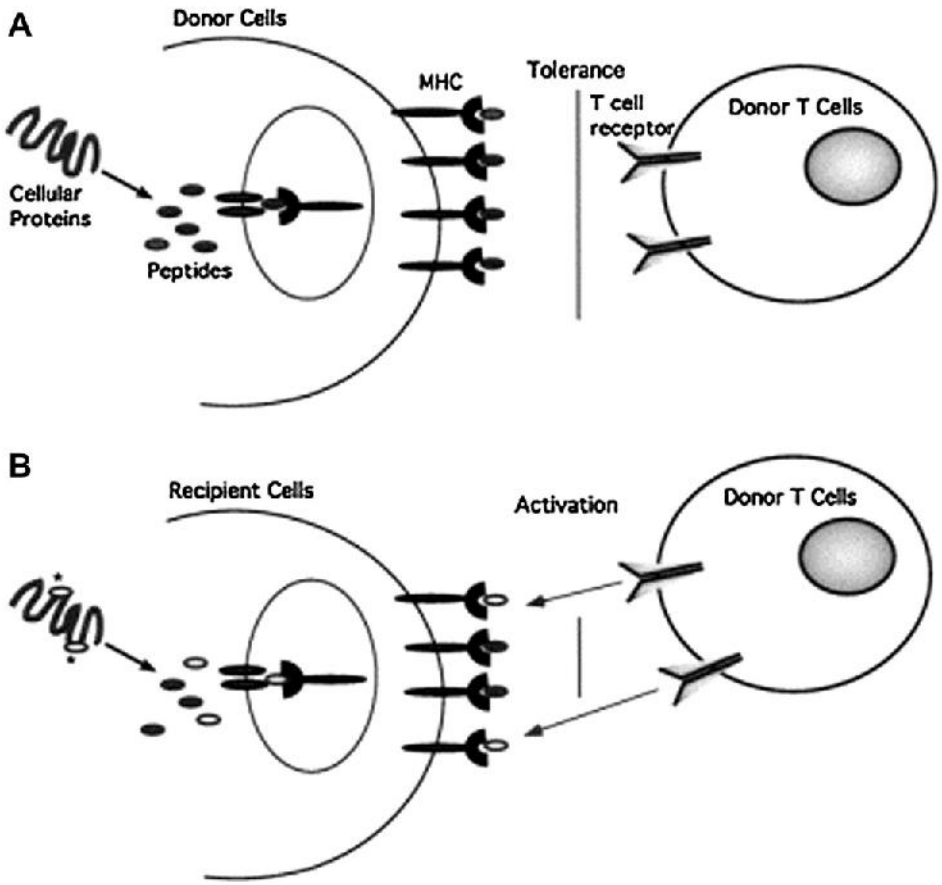

Figure 1.
Minor histocompatibility antigens represent distinct MHC-bound peptides displayed by MHC identical recipient cells. (A) Peptides derived from cellular proteins are displayed on the surface of cells complexed to MHC molecules and autologous T cells are tolerant to these self-peptides. (B) Due to polymorphisms in the genome, cellular proteins expressed by recipient cells may contain amino acid substitutions (depicted by the asterisks) compared with the homologous proteins in donor cells. After processing, these sequences may provide unique peptides that bind to MHC molecules and are displayed at the cell surface. T cells of the donor will recognize the unique peptides on recipient cells as foreign. Reproduced with permission from Riddell SR, Berger C, Murata M, et al. The graft versus leukemia response after allogeneic hematopoietic stem cell transplantation. Blood Rev. 2003 Sep;17(3):153-62.

Nat Immunol. 2008 May;9(5):495-502.

Up on the tightrope: natural killer cell activation and inhibition.

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Natural killer (NK) cells circulate through the blood, lymphatics and tissues, on patrol for the presence of transformed or pathogen-infected cells. As almost all NK cell receptors bind to host-encoded ligands, signals are constantly being transmitted into NK cells, whether they interact with normal or abnormal cells. The sophisticated repertoire of activating and inhibitory receptors that has evolved to regulate NK cell activity ensures that NK cells protect hosts against pathogens, yet prevents deleterious NK cell-driven autoimmune responses. Here I highlight recent advances in our understanding of the structural properties and signaling pathways of the inhibitory and activating NK cell receptors, with a particular focus on the ITAM-dependent activating receptors, the NKG2D-DAP10 receptor complexes and the CD244 receptor system.

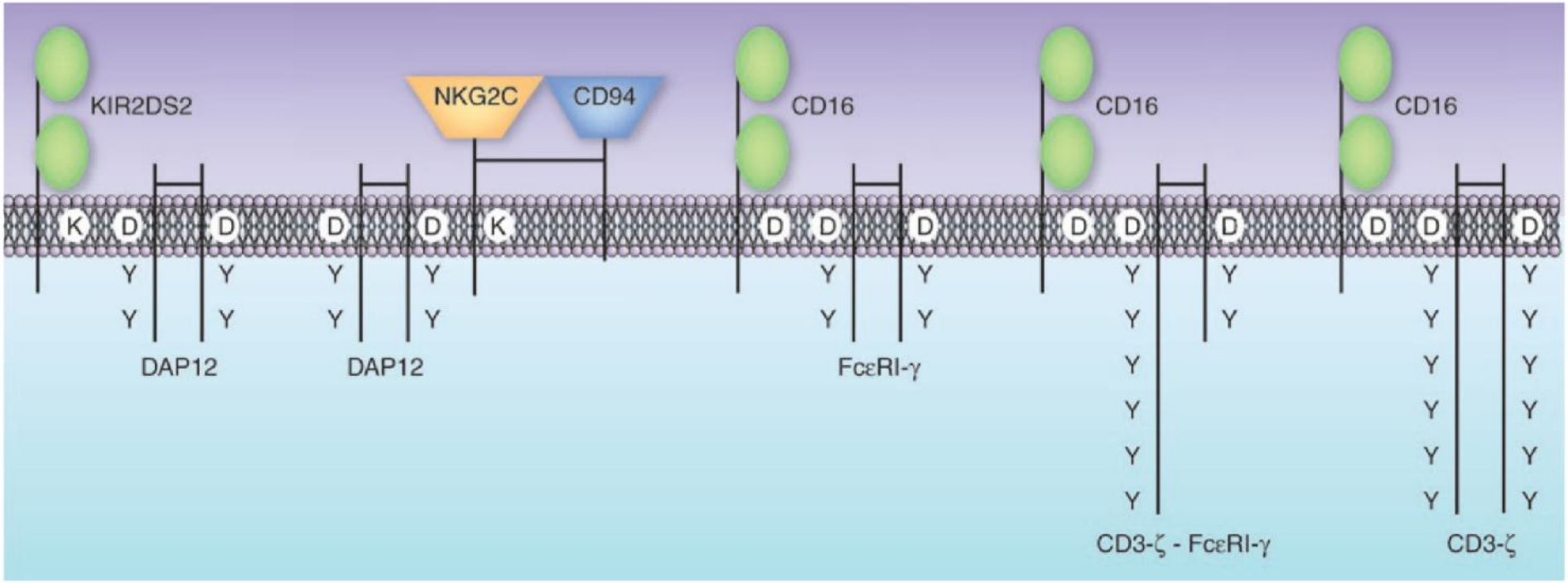

Figure 1.
ITAM-containing NK receptors. Schematic representation of NK receptors of the immunoglobulin superfamily or C-type lectin—like family that pair with the ITAM-bearing DAP12, FcεRI-γ and CD3-ζ signaling subunits. For a comprehensive list of ITAM-signaling NK cell receptors, see Supplementary Table 1. Note that human CD16 can pair with homodimers of FcεRI-γ or CD3-ζ or with heterodimers of FcεRI-γ and CD3-ζ, whereas mouse CD16 signals efficiently only with homodimers of FcεRI-γ. ITAM-bearing signaling subunits contain aspartate residues (D) within their transmembrane segments that associate noncovalently with oppositely charged lysine or arginine residues within the transmembrane of the receptors, an exception being CD16, which also has an aspartate residue within its transmembrane. Y, tyrosine residues within ITAM domains.

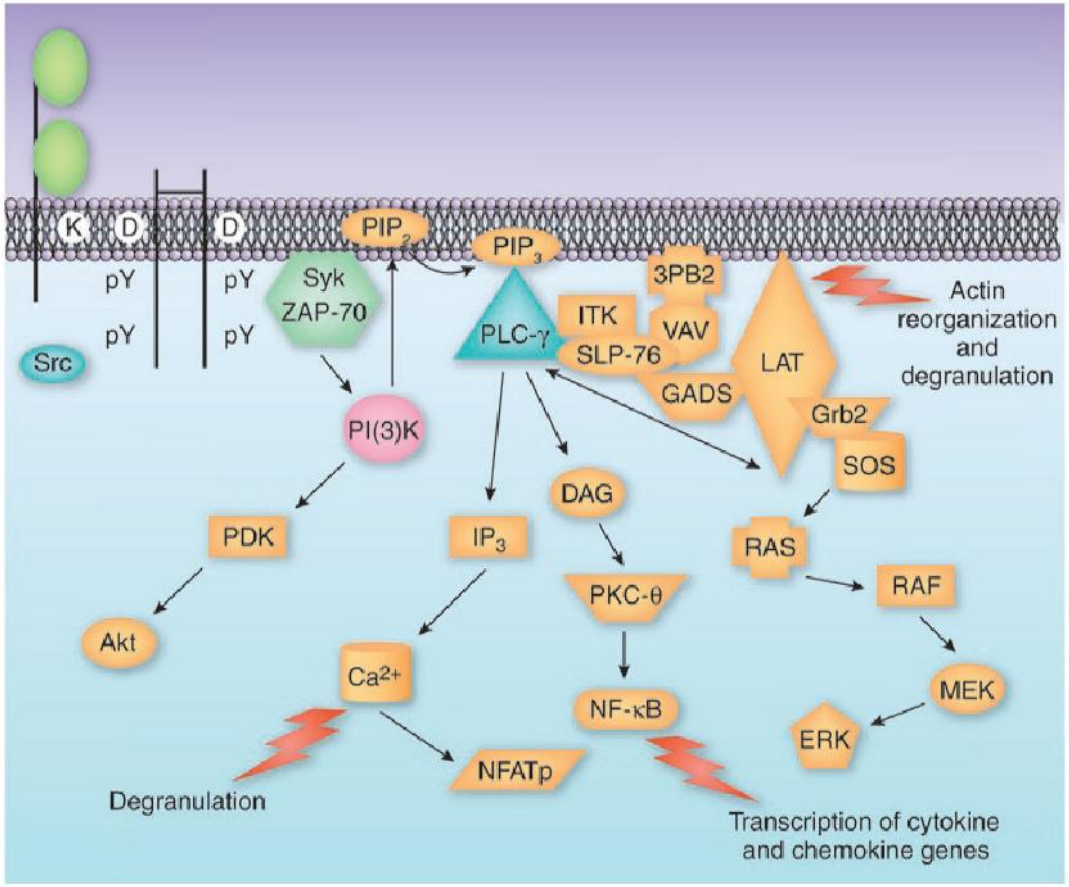

Figure 2.
ITAM-mediated signaling in NK cells. ITAM-bearing signaling subunits are phosphorylated, probably by Src family kinases, after receptor engagement. Syk and/or ZAP-70 (both of which are expressed by human and mouse NK cells) are recruited to the phosphorylated ITAMs, initiating a cascade of downstream signaling as depicted. The signaling pathways depicted are hypothetical and were deduced by synthesizing results from many studies investigating ITAMcoupled receptor signaling in human and mouse NK cells. DAG, diacylglycerol; IP3, inositol-3,4,5-trisphosphate; PIP2, phosphatidylinositol-3,4-bisphosphate; PIP3, phosphatidylinositol-3,4,5-trisphosphate; pY, phosphotyrosine; ITK, tyrosine kinase; GADS and 3BP2, adaptor proteins; NFATp and NF-κB, transcription factors; PDK, phosphoinositidedependent protein kinase; PKC-θ, protein kinase C-θ; RAF, mitogen-activated protein (MAP) kinase kinase kinase; RAS, GTPase.

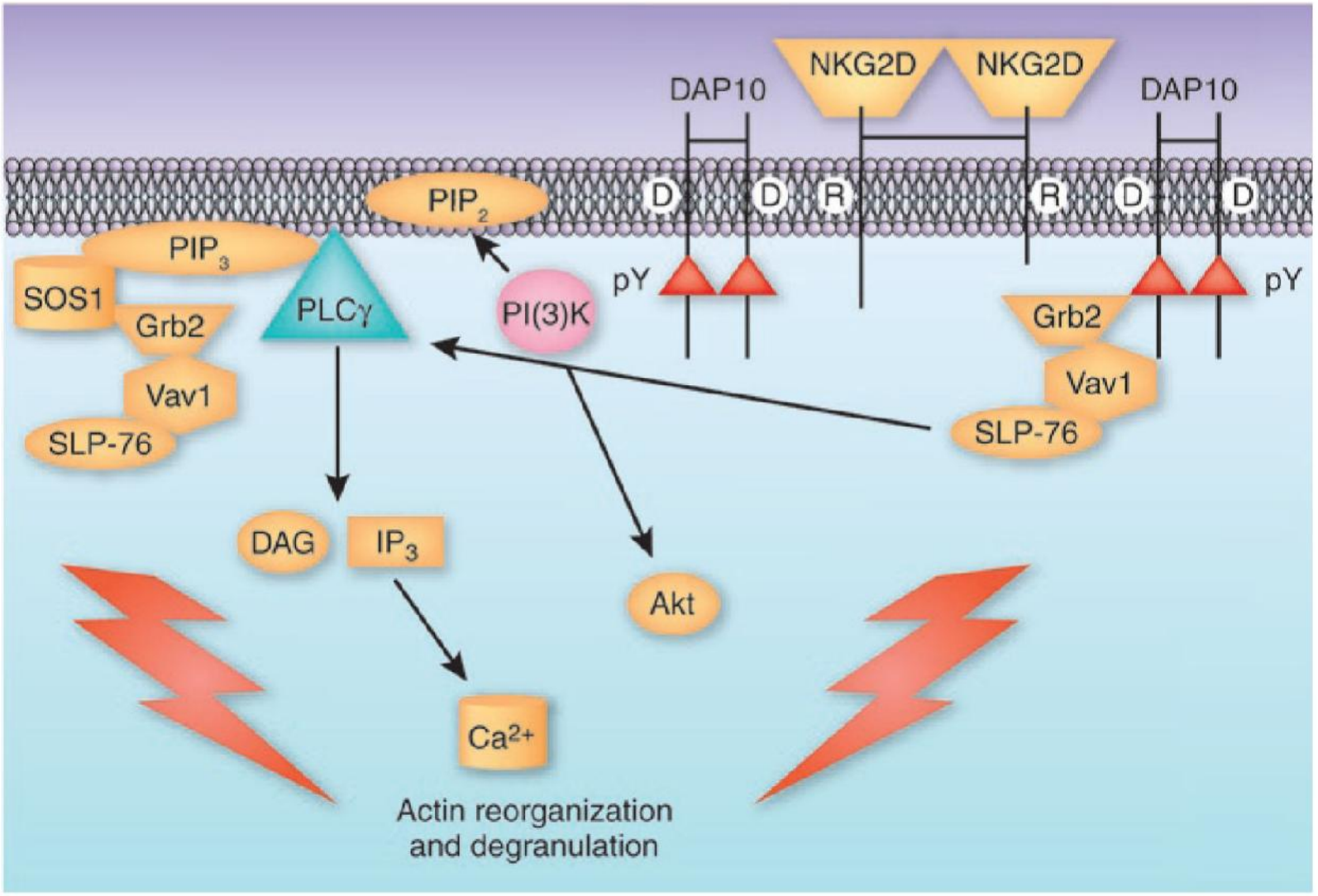

Figure 3.
DAP10-mediated signaling in NK cells. Cross-linking NKG2D causes NK cell activation that involves the recruitment of the p85 subunit of PI(3)K and recruitment of the Grb2-Vav1-Sos1 complex to the phosphorylated YINM motif in the cytoplasmic domain of DAP10. These events trigger distal signaling cascades as depicted.

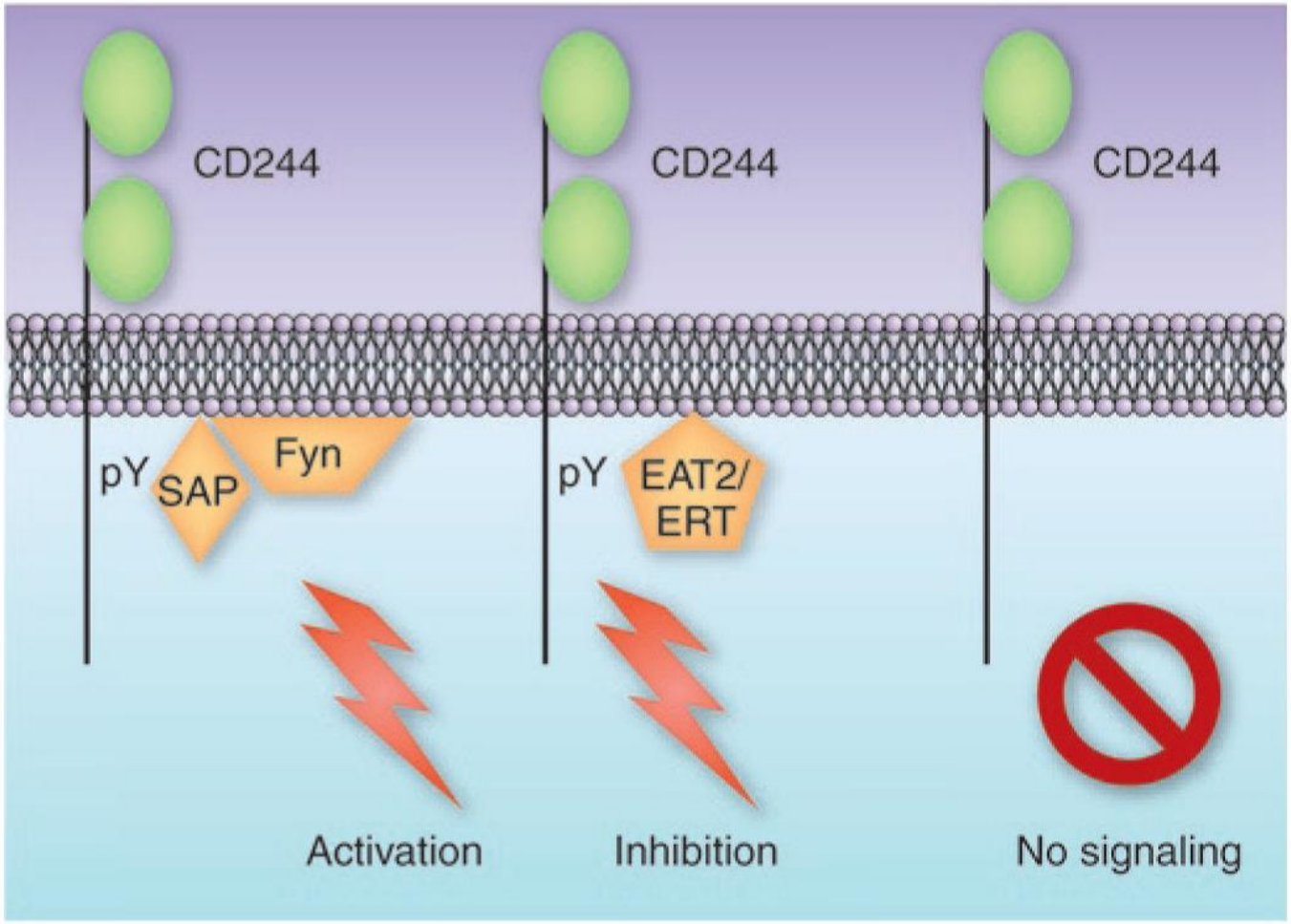

Figure 4.
CD244 receptor complexes in NK cells. Phosphorylation of the tyrosines in the TIYXX(V/I) motifs in the cytoplasmic domain of CD244 can recruit the adaptor proteins SAP, EAT2 or ERT (ERT exists in mice, but not humans). SAP binds to Fyn to mediate signal transduction. It has been proposed that the CD244-SAP–Fyn complex is responsible for NK cell activation when NK cells encounter target cells expressing CD48, a ligand of CD244. Alternatively, evidence suggests that CD244-EAT2 and CD244-ERT complexes deliver inhibitory signals into NK cells, although this remains controversial44,45,56,61.

Blood. 2008 Aug 1;112(3):461-9.

Human natural killer cells.

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Natural killer (NK) cells were discovered more than 30 years ago. NK cells are large granular lymphocytes that belong to the innate immune system because unlike T or B lymphocytes of the adaptive or antigen-specific immune system, NK cells do not rearrange T-cell receptor or immunoglobulin genes from their germline configuration. During the past 2 decades there has been a substantial gain in our understanding of what and how NK-cells "see," lending important insights into their functions and purpose in normal immune surveillance. The most recent discoveries in NK-cell receptor biology have fueled translational research that has led to remarkable results in treating human malignancy.

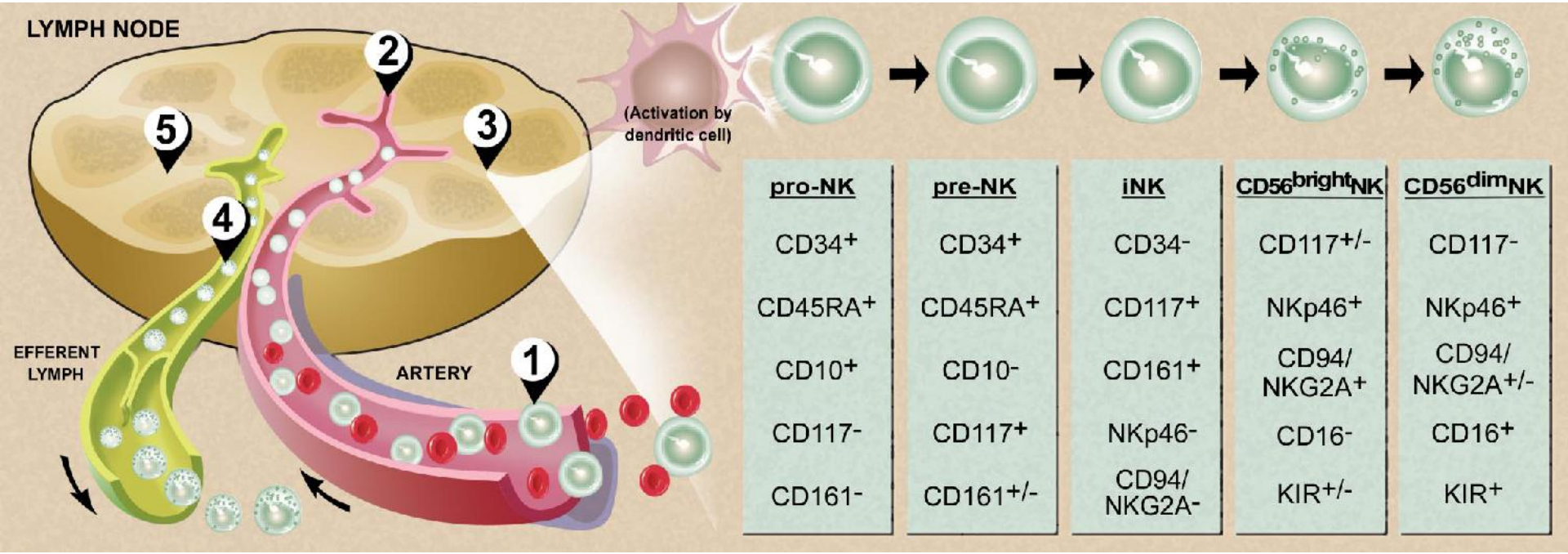

Figure 2. Model of human NK-cell development. (1) Bone marrow–derived CD34CD45RA HPCs circulate in the blood and (2) extravasate across lymph node high
endothelial venules to enter the parafollicular space. There, (3) pro-NK cells are activated to progress through distinct stages of maturation (far right) to create both CD56bright
and CD56dim NK cells.31 Maturing CD56dim NK cells return to the circulation via the efferent lymph (4),32 whereas some CD56bright NK cells remain within the secondary lymphoid
tissue to interact with DCs (5).21,23,33,34 Illustration by Debra T. Dartez.

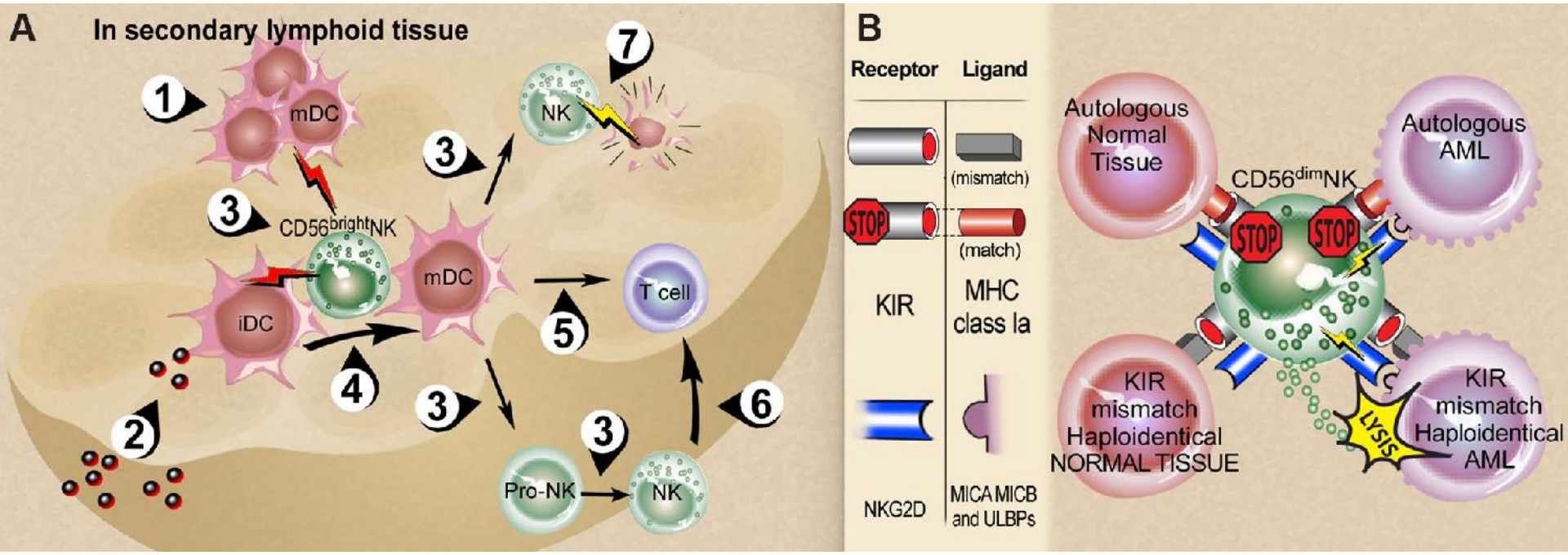

Figure 3. CD56bright and CD56dim NK-cell interactions. (A) NK-DC interactions in secondary lymphoid tissue (SLT). (1) Activated mature DCs (mDCs) enter SLT from periphery or (2) immature DCs (iDCs) receive pathogens within SLT. Each express and/or secrete a variety of cytokines (3) that are required for NK-cell maturation and survival (eg, DC IL-15) and NK cell proinflammatory cytokine production (eg, DC IL-12 in combination with DC IL-1, IL-15, IL-18). Activated CD56bright NK cells in turn secrete TNF- and GM-CSF that contribute to DC maturation, (4) and IFN- that contributes to DC activation and thus indirectly to antigen-specific T-cell priming (5). NK-cell IFN- also contributes directly to T-cell priming (6). NK cells can kill immature autologous DCs (7) via NKp30, which may assist in editing out hyporesponsive DCs or by limiting T-cell priming.33,34 (B) Summary of NK-cell recognition. The functional consequences of NK-cell receptor recognition depend on the integration of both inhibition and activation signals received in response to engagement of target cell ligands.58,59 Upper left: normal autologous tissues are not attacked because the predominant signal is recognition of self-MHC class Ia ligands by inhibitory KIRs (and other inhibitory receptors such as NKG2A/CD94 recognizing their ligands, not shown) in the absence of ligands for activating NK receptors. Upper right: Malignant autologous tumors such as acute myeloid leukemia (AML) have high-density surface expression of classical MHC class Ia and nonclassical MHC class I that that bind to KIR and NKG2A/CD94, respectively, and dominate over engagement of NK-cell activation receptors with their cognate ligands. Lower left: Normal allogeneic host tissue presumably lacks ligands that engage dominant activating NK receptors such as NKG2D and NCR, despite a mismatch of donor NK KIR with host MHC class Ia as well as donor NKG2A/CD94 and host HLA-E (not shown). Lower right: A mismatch of donor NK KIR and host MHC class Ia in the presence of ligand-engaged NKG2D, NCR, and other NK activation receptors60 likely contributes the dominant NK response of target cell lysis.48,61,62 Illustration by Debra T. Dartez.

Michael A. Caligiuri

Twenty-five years ago, I was “on call” as an uninspired third-year medical student at Stanford, and admitted a kidney transplant patient with renal failure secondary to acute rejection.We tried using an experimental drug called cyclosporine to see if, in the words of my resident, “we could trick the patient’s T cells into thinking the renal graft was not foreign.” Soon, the patient was urinating again. That moment was like a lightning bolt for me: I saw the application of basic pharmacology to clinical medicine in the setting of transplantation immunology. From that day on, I knew that the application of basic immunology to the field of clinical transplantation was where I was going. Once I found out you needed to be a surgeon to transplant most tissues, the idea of bone marrow transplantation for hematologic malignancies became very, very appealing! It is gratifying to see that after a quarter of a century, the secrets of natural killer cell receptor biology are being revealed and quickly applied to cure cancer in the setting of allogeneic bone marrow transplantation. For the students: When my kids tell me I work hard, I tell them I get paid well to do my hobby. Find and pursue your passion. The rewards will follow.

Immunol Rev. 2008 Aug;224:70-84.

Negative signaling by inhibitory receptors: the NK cell paradigm.

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Receptors carrying immunoreceptor tyrosine-based inhibition motifs (ITIMs) in their cytoplasmic tail control a vast array of cellular responses, ranging from autoimmunity, allergy, phagocytosis of red blood cells, graft versus host disease, to even neuronal plasticity in the brain. The inhibitory function of many receptors has been deduced on the basis of cytoplasmic ITIM sequences. Tight regulation of natural killer (NK) cell cytotoxicity and cytokine production by inhibitory receptors specific for major histocompatibility complex class I molecules has served as a model system to study the negative signaling pathway triggered by an ITIM-containing receptor in the physiological context of NK-target cell interactions. Advances in our understanding of the molecular details of inhibitory signaling in NK cells have provided a conceptual framework to address how ITIM-mediated regulation controls cellular reactivity in diverse cell types.

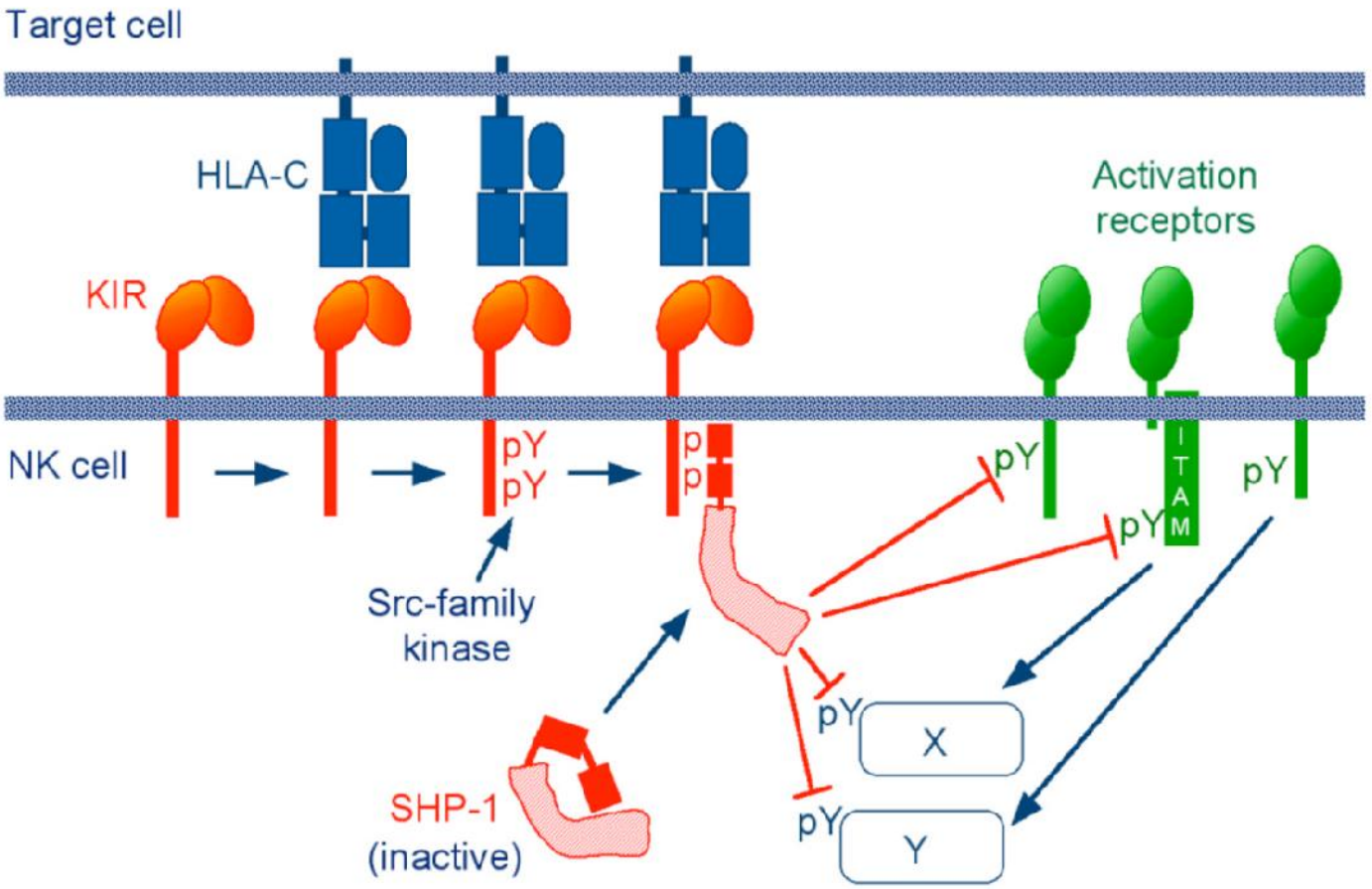

Fig. 1. Early model for inhibitory signalling by KIR in NK cells
Sequential steps in the inhibition of NK cells by KIR: Binding of inhibitory KIR to HLA-C on target cells; KIR clustering; phosphorylation of two tyrosines within cytoplasmic ITIM sequences; recruitment and activation of the tyrosine phosphatase SHP-1 to the tyrosinephosphorylated ITIMs; dephosphorylation of multiple substrates, such as activation receptors and signalling molecules (*X, Y*) by catalytically active SHP-1. The Src-family kinase that phosphorylates the ITIMs may be provided in *trans* by activation receptors. Inhibitory molecules are indicated in *red*, activation receptors in *green*.

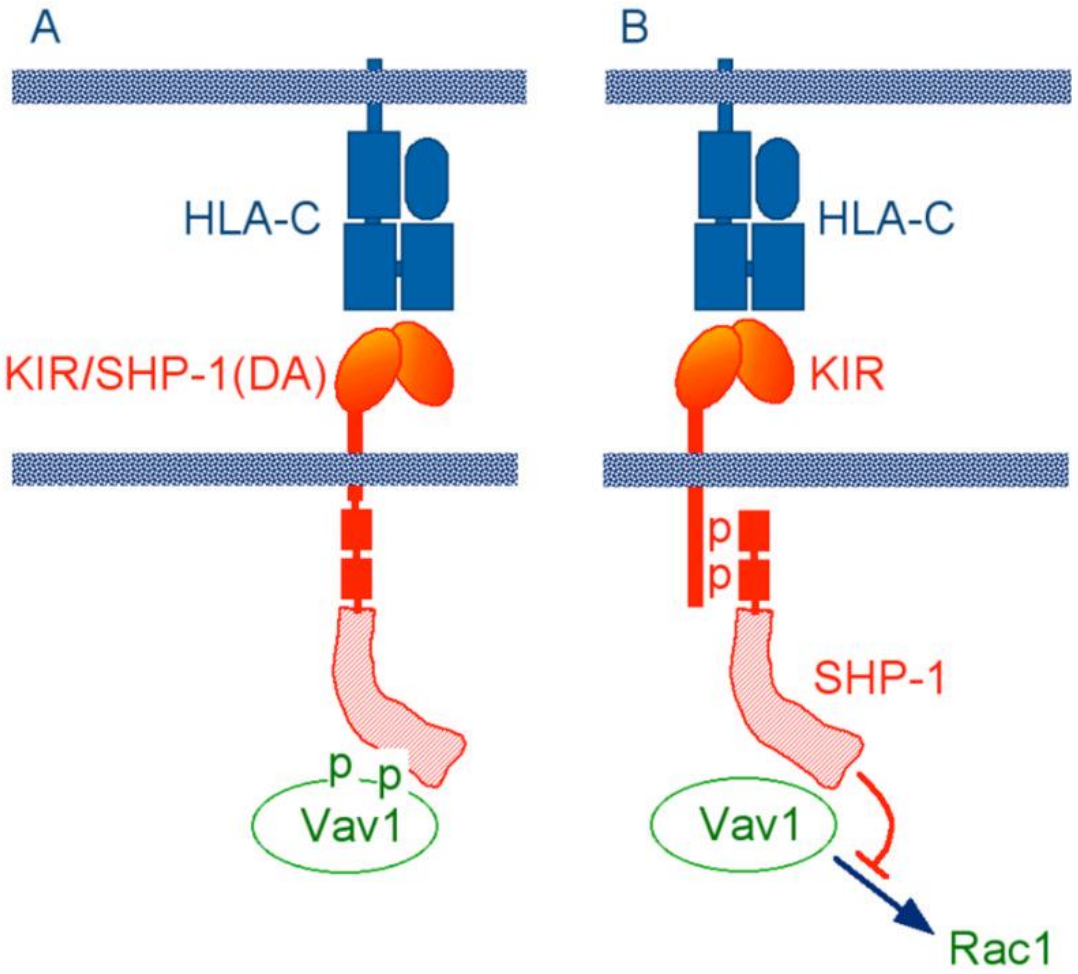

Fig. 2. Identification of Vav1 as the predominant substrate during inhibition of NK cells by KIR
(A) Tyrosine-phosphorylated Vav1 was “trapped” by a chimeric KIR/SHP-1 receptor during inhibition of YTS NK cells by target cells expressing an HLA-C ligand of KIR. The trap was generated by an Asp to Ala mutation (DA) in the SHP-1 catalytic site, and by the fusion of SHP-1(DA) to the KIR cytoplasmic tail. (B) Vav1 trapping, as shown in panel *A*, implies that catalytically active SHP-1 recruited by KIR during inhibition blocks NK cell activation through
dephosphorylation of Vav1, which prevents the guanine exchange factor activity of Vav1 towards the GTPase Rac1.

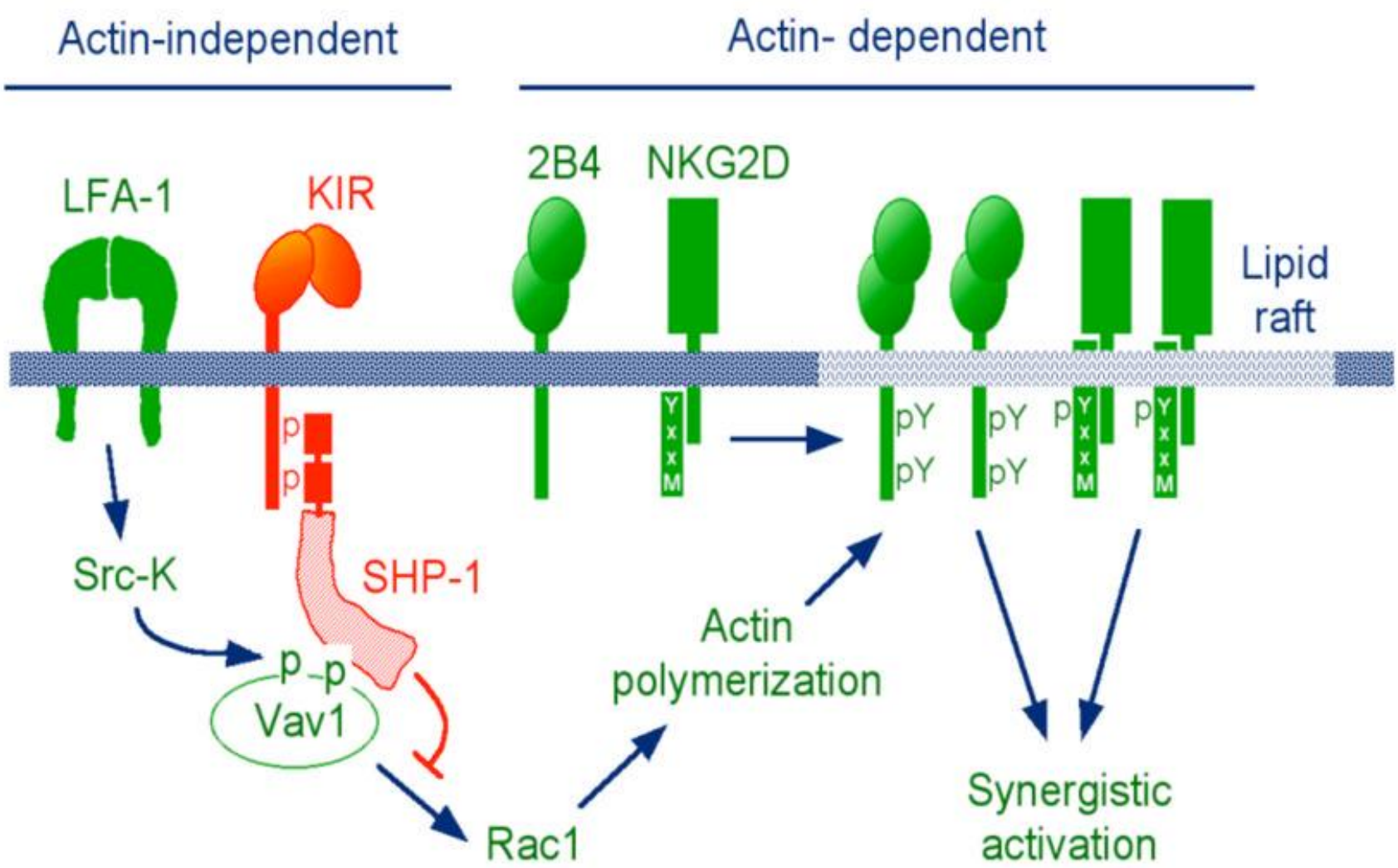

Fig. 3. Revised model for inhibitory signalling by KIR in NK cells
Early, actin-independent signalling by LFA-1 phosphorylates and activates Vav1. Actinindependent dephosphorylation of Vav1 by ITIM–bound SHP-1 prevents actin-dependent processes, such as recruitment of natural cytotoxicity receptors (e.g. NKG2D and 2B4) to lipid rafts, receptor tyrosine phosphorylation, and synergistic signalling by co-activation receptors.

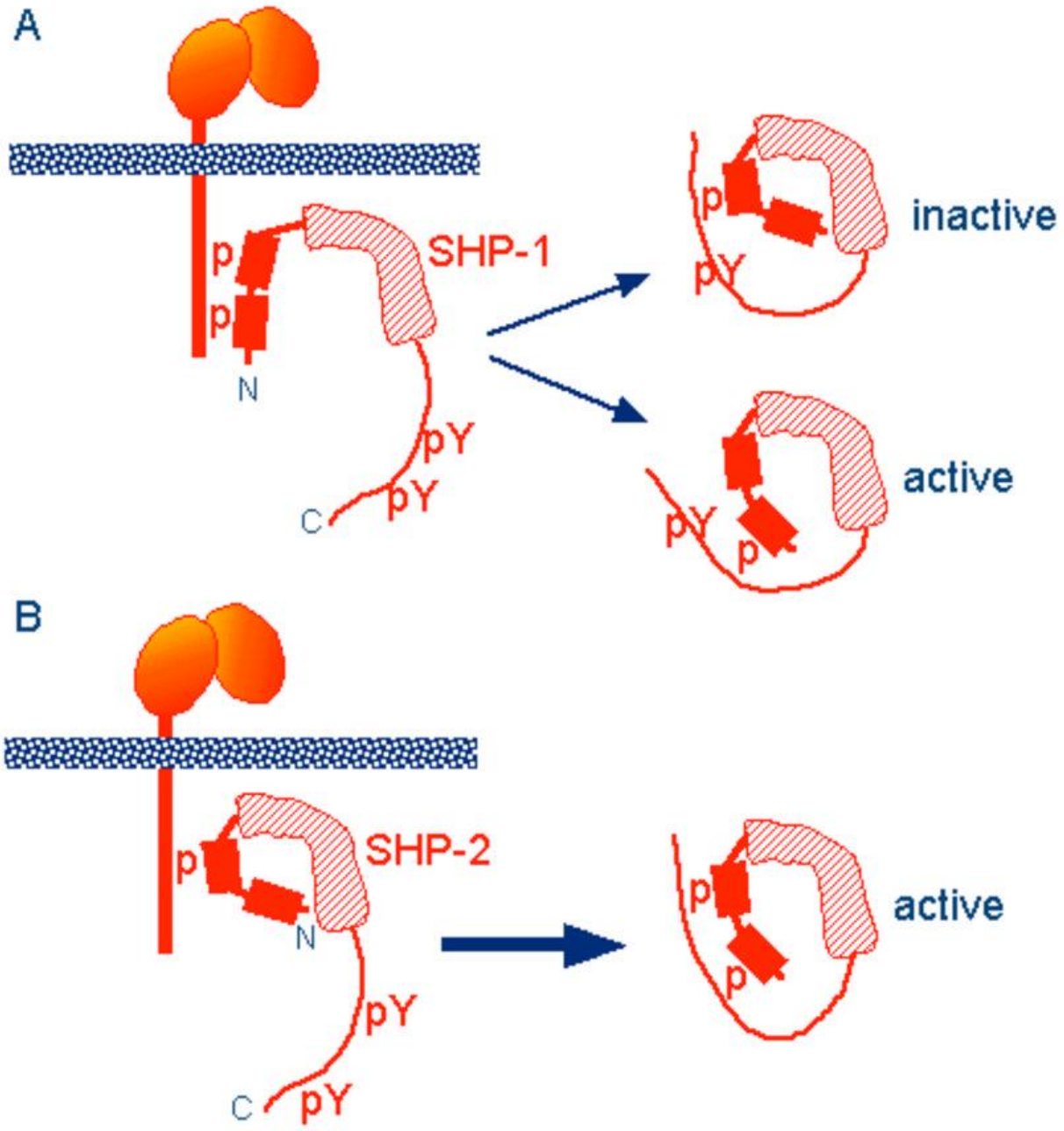

Fig. 4. Distinct structural properties of SHP-1 and SHP-2 suggest different inhibitory potential

These diagrams are adapted from (86) and (85). (A) The preferential binding of phosphorylated ITIM to the second SH2 domain of SHP-1 (1,4), and the crystal structure of SHP-1 (107) suggest that the first ITIM of inhibitory KIR binds to the second SH2 domain of SHP-1.

The two C-terminal tyrosines of SHP-1 can engage in intramolecular interactions with the SH2 domains, when phosphorylated. However, the short spacing (28 amino acids) between the tyrosines preclude intramolecular binding to both SH2 domains simultaneously. (B) SHP-2 phosphorylated at both C-terminal tyrosines (38 amino acids apart) can form a divalent, intramolecular complex with its own SH2 domains, which retains catalytic activity. Unlike SHP-1, which requires phosphorylation of both ITIMs for binding, SHP-2 binds to either both phosphorylated ITIMs or to the first phosphorylated ITIM only, as indicated.

Curr Opin Cell Biol. 2008 Oct;20(5):597-605. Epub 2008 Jul 17.

The killer's kiss: the many functions of NK cell immunological synapses.

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Natural killer (NK) cells comprise a subset of lymphocytes involved in protection against microbial pathogens and tumors. NK cells recognize host cells that are missing MHC class I molecules and eliminate them through localized delivery of lytic granules. The majority of NK cell effector functions require direct cell-to-cell contact. Binding to a target cell is accompanied by creation of complex structures at the cell-cell interface known as immunological synapses. Recent studies have contributed immensely to the characterization of several types of NK cell immunological synapses and understanding of the variety of processes originating at this intriguing place. The emerging picture illustrates NK cell immune synapses as the sites of highly complex regulation of NK cell activity.

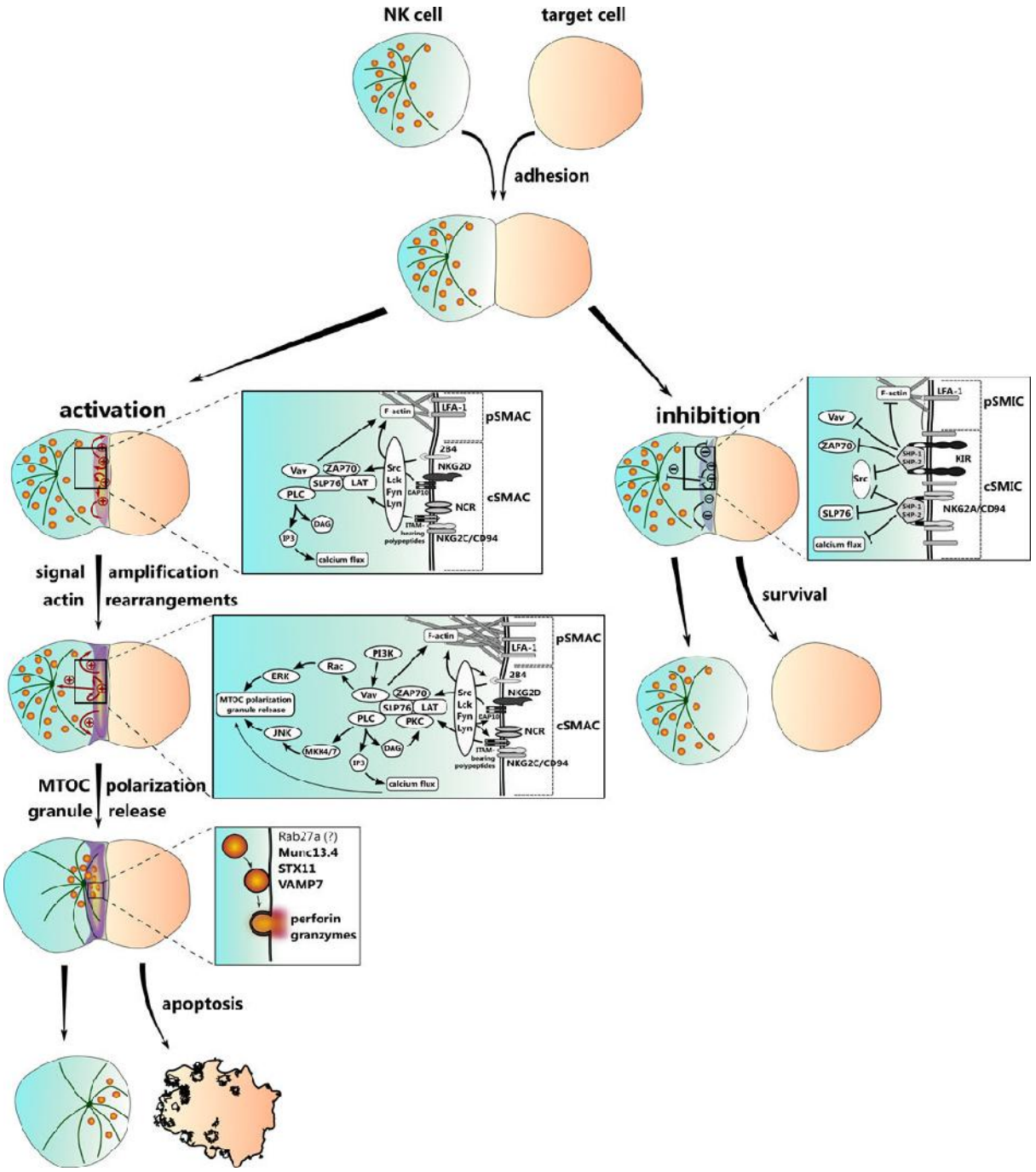
NK cell
target cell
adhesion
activation
inhibition
signal
actin
amplification
rearrangements
MTOC
granule
polarization
release
apoptosis
survival
pSMAC
cSMAC
pSMIC
cSMIC
F-actin
LFA-1
Vav
ZAP70
SLP76
LAT
PLC
DAG
IP3
calcium flux
Src
Lck
Fyn
Lyn
NKG2D
NCR
NKG2C/CD94
KIR
NK62A/CD94
PI3K
Rac
ERK
JNK
MKK4/7
PKC
MTOC polarization
granule release
Rab27a (?)
Munc13.4
STX11
VAMP7
perforin
granzymes

Figure 1. The NK cell immunological synapse is formed in distinct stages
The encounter between the NK and a target cell results in adhesion and conjugate formation (top). The balance between activating and inhibitory receptor signaling on the cell-cell interface decides the outcome of the interaction. The lack of MHC I molecules on the target cell, caused by viral infection or tumorigenesis, favors formation of the activating NKIS (left). Engagement of NK cell activating receptors by their ligands induces phosphorylation of membrane proximal signaling molecules and initial wave of actin cytoskeleton rearrangements. This, in turn, leads to more stable conjugation by creation of an F-actin ring in the pSMAC area and formation of a signalosome comprised of many signaling and adapter molecules in the cSMAC. Thus, a positive feedback loop is generated causing signal amplification and sustained signaling that stimulates robust actin polymerization and polarization of the MTOC to the activating NKIS. Lytic granules, containing perforin and granzymes, are transported along microtubule tracks and with MTOC translocation they are delivered to the cSMAC, where they are subsequently released. Perforin makes pores in the membrane of target cell, allowing granzymes to enter the cell and induce apoptosis. After induction of target cell lysis, the NK cell detaches from its target and can search for another target. Conversely, the presence of MHC I on the surface of target cell results in ligation of NK cell receptors that are capable of dominant inhibitory signaling and formation of the inhibitory NKIS (right). Engagement of inhibitory receptors leads to quick disruption of activation signaling by phosphatase-mediated dephosphorylation of membrane proximal signaling molecules (or even possibly macromolecular structures) and blocking of the signalosome formation. This prevents large scale actin cytoskeleton rearrangements and inhibits MTOC and lytic granule polarization, resulting in survival of the target cell. The diagrams represent only selected molecules. The drawings are not to scale.

Blood. 2009 Jan 15;113(3):726-32. Epub 2008 Oct 22.

Donors with group B KIR haplotypes improve relapse-free survival after unrelated hematopoietic cell transplantation for acute myelogenous leukemia.

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Survival for patients with acute myeloid leukemia (AML) is limited by treatment-related mortality (TRM) and relapse after unrelated donor (URD) hematopoietic cell transplantation (HCT). Natural killer (NK)-cell alloreactivity, determined by donor killer-cell immunoglobulin-like receptors (KIRs) and recipient HLA, correlates with successful HCT for AML. Hypothesizing that donor KIR genotype (A/A: 2 A KIR haplotypes; B/x: at least 1 B haplotype) would affect outcomes, we genotyped donors and recipients from 209 HLA-matched and 239 mismatched T-replete URD transplantations for AML. Three-year overall survival was significantly higher after transplantation from a KIR B/x donor (31% [95% CI: 26-36] vs 20% [95% CI: 13-27]; P = .007). Multivariate analysis demonstrated a 30% improvement in the relative risk of relapse-free survival with B/x donors compared with A/A donors (RR: 0.70 [95% CI: 0.55-0.88]; P = .002). B/x donors were associated with a higher incidence of chronic graft-versus-host disease (GVHD; RR: 1.51 [95% CI: 1.01-2.18]; P = .03), but not of acute GVHD, relapse, or TRM. This analysis demonstrates that unrelated donors with KIR B haplotypes confer significant survival benefit to patients undergoing T-replete HCT for AML. KIR genotyping of prospective donors, in addition to HLA typing, should be performed to identify HLA-matched donors with B KIR haplotypes.

Immunology. 2009 Mar;126(3):423-35. Epub 2008 Sep 5.

Characterization of the recognition and functional heterogeneity exhibited by cytokine-induced killer cell subsets against acute myeloid leukaemia target cell.

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The polyclonal cytokine-induced killer (CIK) cells exhibit potent cytotoxicity against a variety of tumour cells including autologous and allogeneic acute myeloid leukaemic (AML) targets. At maturity, three lymphocyte subsets: CD3(-) CD56(+), CD3(+) CD56(-) and CD3(+) CD56(+), constitute the bulk of the CIK cell culture. The CD3(-) CD56(+) subset behaves like classical natural killer (NK) cells where cytotoxicity is potentiated by blocking the human leucocyte antigen Class I molecules in the AML targets. Both the CD3(+) CD56(+) and CD3(+) CD56(-) subsets, though known to kill autologous and allogeneic targets to a comparable degree and therefore non-major histocompatibility complex (MHC)-restricted, nevertheless require the presence of the MHC molecule on the target, which interacts with their CD3-T-cell receptor complex. Although CIK cells are often termed 'NK-like' T cells, we have demonstrated that the well-characterized NK receptors KIR, NKG2C/E, NKG2D and DNAM-1 are not involved in the process of AML recognition for the CD3(+) CD56(-) and CD3(+) CD56(+) subsets. The CD3(+) CD56(+) and CD3(+) CD56(-) subsets express a polyclonal and comparable TCRVbeta repertoire in a Gaussian distribution. The CD3(+) CD56(+) subset kills AML targets more efficiently than its CD3(+) CD56(-) counterpart because of the presence of a higher proportion of CD8(+) cells. The CD3(+) CD56(+) subset comprise more terminally differentiated late effector T cells that bear the CD27(+) CD28(-) or CD27(-) CD28(-) phenotype, with a higher granzyme A content. In comparison, the phenotype of the CD3(+) CD56(-) subset is consistent with early effector T cells that are CD27(+) CD28(+) and CD62L(+), known to be less cytotoxic but possess greater proliferative potential.

Korean J Lab Med. 2009 Apr;29(2):89-96.

Expansion and activation of natural killer cells for cancer immunotherapy.

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Natural killer (NK) cells can kill a wide range of cancer cells and are a promising tool for cell therapy of cancer. NK cells cytotoxicity is regulated by a balance between stimulatory and inhibitory signals. Interleukin-2 is known to increase NK cell cytotoxicity. Although many cytokines have been studied in efforts to induce durable NK cell expansions, most reports indicate a rather modest effect and the requirement for additional stimuli. We found that contact with the K562 myeloid leukemia cell line, genetically modified to express a membrane-bound form of interleukin-15 and the ligand for the costimulatory molecule 4-1BB, induced vigorous expansion of NK cells from peripheral blood. Based on these findings, we developed a method for large-scale clinical-grade expansion of NK cells. This method is currently used to expand allogeneic NK cells for infusion in patients with leukemia and solid tumors. We here summarize methods for expansion and activation of NK cells from human peripheral blood mononuclear cells as well as clinical-scale methods to produce NK cells for immunotherapy under Current Good Manufacturing Practices (cGMP) conditions.

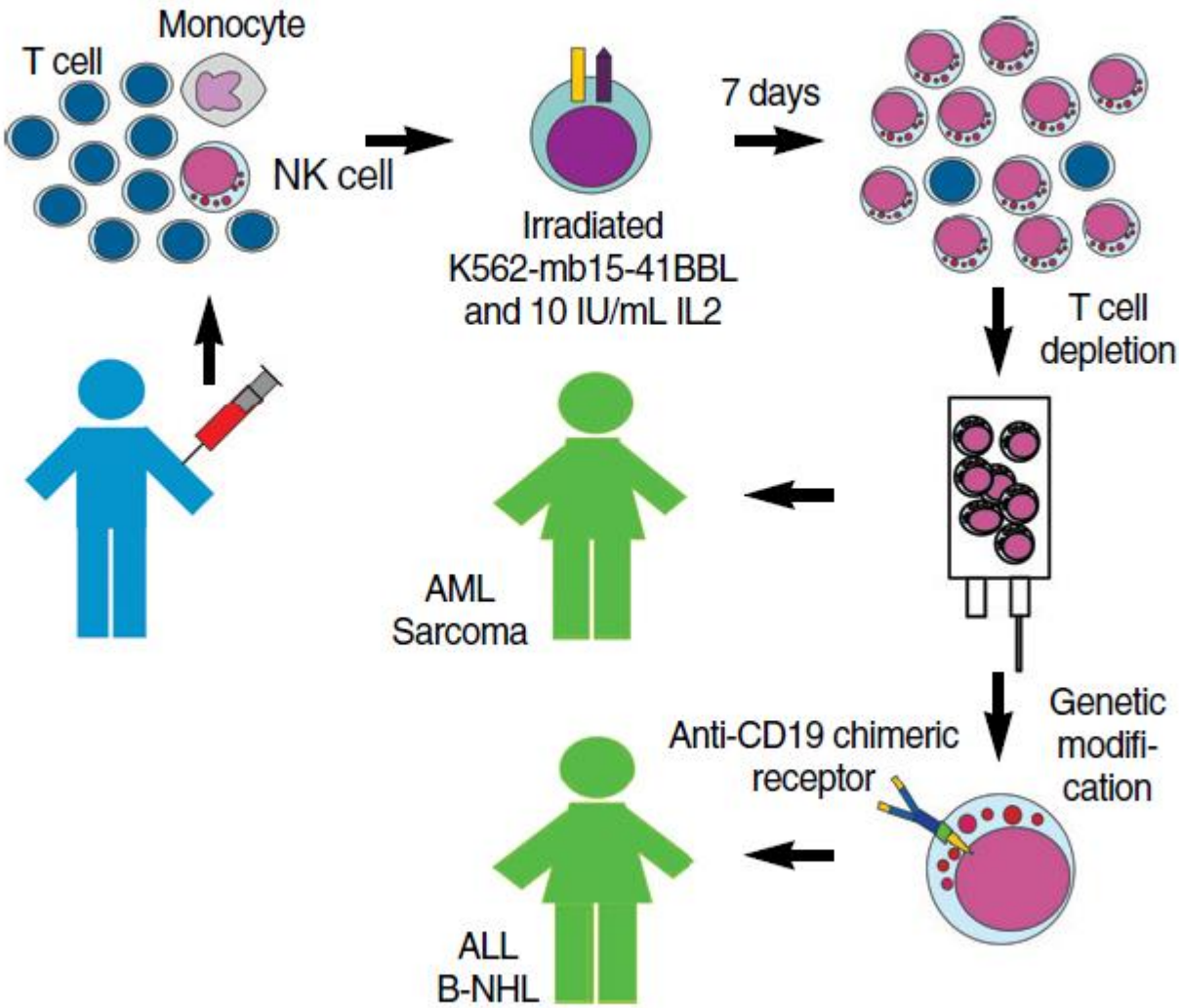

Fig. 1. Schematic representation of protocols using expanded NK cells at St Jude Children's Research Hospital. The leukapheresis product obtained from a haploidentical donor is mixed with irradiated K562-mb15-41BBL cells. After 7 days of culture, most cells recovered are activated NK cells. After T-cell depletion using the CliniMACS system, NK cells are infused in patients with NK-sensitive malignancies such as acute myeloid leukemia (AML), Ewing sarcoma or rhabdomyosarcoma. For patients whose neoplasia is less sensitive to NK cytotoxicity, such as B-lineage acute lymphoblastic leukemia (ALL) or B-cell non-Hodgkin lymphoma (BNHL), expanded NK cells are transduced with an anti-CD19 chimeric receptor before infusion.

Curr Opin Immunol. 2009 Oct;21(5):525-30. Epub 2009 Aug 28.

Natural killer cell allorecognition of missing self in allogeneic hematopoietic transplantation: a tool for immunotherapy of leukemia.

Velardi A, Ruggeri L, Mancusi A, Aversa F, Christiansen FT.

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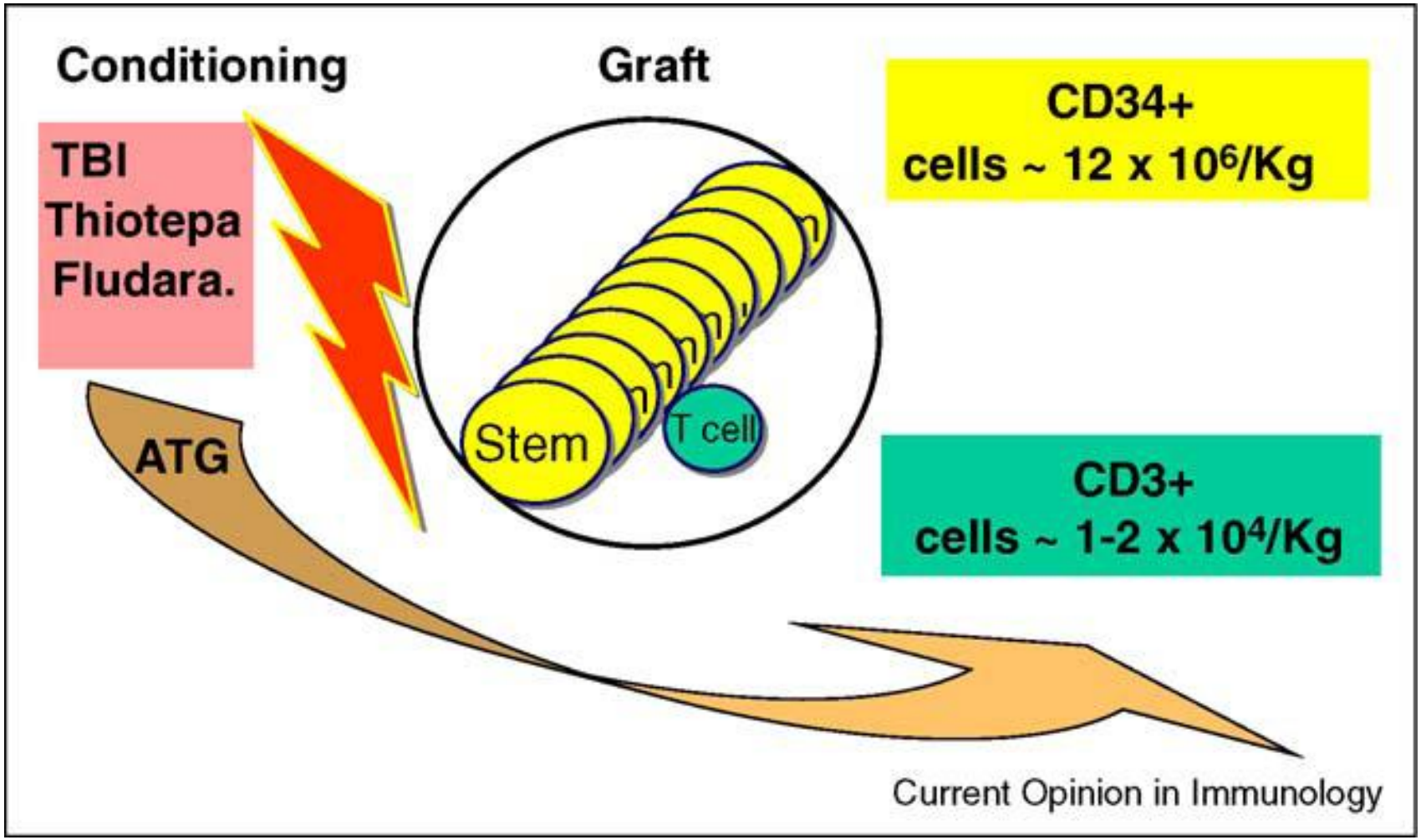
Donor-versus-recipient natural killer (NK) cell alloreactivity has been established as a key therapeutic element in HLA haplotype mismatched hematopoietic transplants in adult AML and pediatric ALL and as a possible beneficial effector in cord blood transplant for AML. It is effected by functional NK cells which express inhibitory killer cell immunoglobulin-like receptor(s) (KIR) for self-class I ligand(s), sense missing expression of donor KIR ligand(s) in the recipient and mediate alloreactions. At present NK cell allotherapy for leukemia is deployed through stem cell transplantation (and ensuing NK cell reconstitution) across KIR ligand mismatches. Studies have been performed to infuse NK cells for immunotherapy outside the fields of transplantation and/or harness the function of endogenous NK cells in patients with hematological malignancies.



The protocol for HLA haploidentical transplantation for acute leukemia as designed by Aversa et al. [4]. Conditioning consists of 8 Gy total-body irradiation on day 9 before transplant in a single fraction at an instantaneous dose-rate of 0.16 Gy/min; lungs shielded to receive 0.04 Gy; thiotepa (5 mg/kg daily) on days 8 and 7; fludarabine (40 mg/m2 daily) from day 7 to day 3; rabbit antithymocyte globulin (ATG) at 5 mg/kg daily from days 5 to 2. The graft contains 12 106 CD34+ cells and 1–2 104 CD3+ cells/kg body weight. Ex vivo T cell depletion of the graft combined with in vivo T depletion exerted by ATG prevents GvHD, without need of post-transplant pharmacological immune suppression. The stem cell ‘megadose’ ensures engraftment across HLA barriers.

Post-transplant regeneration of donor-versus-recipient-alloreactive NK cell repertoire. Left: in donors, NK cells which express inhibitory KIRs for self- HLA ligands are functionally active as they become ‘licensed/educated’ upon interaction with self-HLA molecules and thus enabled to exert alloreactivity against mismatched allogeneic targets which do not express self-HLA KIR ligands. In this example, a donor NK cell expressing KIR2DL2/ 3, inhibiting receptor for the self-HLA-C Group 1 allele, does not find this allele group in the recipient and is activated to kill the recipient target. Right: engrafted stem cells from the KIR ligand-mismatched donor give rise to the exact same donor HLA-licensed/educated repertoire, including alloreactive clones. Alloreactive NK cells eradicate leukemia, prevent rejection by killing recipient T lymphocytes and GvHD by killing recipient-type dendritic cells. NK cell alloreactivity does not attack other tissues as it does not cause GvHD [7–12].

Current Opinion in Immunology

Haematologica. 2009 Nov;94(11):1590-4. Epub 2009 Jul 16.

Human acute myeloid leukemia CD34+CD38- stem cells are susceptible to allorecognition and lysis by single KIR-expressing natural killer cells.

Langenkamp U, Siegler U, Jörger S, Diermayr S, Gratwohl A, Kalberer CP, Wodnar-Filipowicz A.

Experimental Hematology, Department of Biomedicine, University Hospital Basel, Basel, Switzerland.

The concept of tumor immunosurveillance has raised prospects for natural killer cell-based immunotherapy of human cancer. The cure of acute myeloid leukemia may depend on eradication of leukemic stem cells, the self-renewing component of leukemia. Whether natural killer cells can recognize and lyse leukemic stem cells is not known. To develop strategies that effectively target acute myeloid leukemia-leukemic stem cells, we investigated anti-leukemic effects of human alloreactive single KIR(+) natural killer cells. Natural killer effectors with KIR specificity mismatched with respect to HLA class I allotype of target cells effectively recognized acute myeloid leukemia-leukemic stem cells defined phenotypically as CD34(+)CD38(-), while healthy bone marrow-derived CD34(+)CD38(-) hematopoietic stem cells were spared, as demonstrated by cytotoxicity and hematopoietic colony-forming assays. The HDAC inhibitor valproic acid increased the activating NKG2D ligand-dependent lysis of acute myeloid leukemia-CD34(+)CD38(-) leukemic stem cells. These results show that alloreactive natural killer cells have the potential to detect and target leukemic stem cells, and thus to improve the treatment outcome in acute myeloid leukemia.

Bone Marrow Transplant. 2010 Feb 22. [Epub ahead of print]

Natural killer-cell KIR repertoire reconstitution after haploidentical SCT.

Stern M, de Angelis C, Urbani E, Mancusi A, Aversa F, Velardi A, Ruggeri L.
Division of Hematology and Clinical Immunology, Department of Clinical and Experimental Medicine, University of Perugia, Perugia, Italy.

We studied killer-cell Ig-like receptor (KIR)/natural killer (NK)-cell group-2-Ag repertoires on donor-derived NK cells in 28 patients after haploidentical SCT in the first 6 months after SCT and correlated results with EFS. The reconstitution hierarchy of potentially alloreactive, single KIR+ NK cells was the following: HLA-C1 binding>HLA-Bw4 binding>HLA-C2 binding. The differences in reconstitution kinetics of the three potentially alloreactive NK cell subsets prompted an updated analysis of EFS in AML patients transplanted from haploidentical donors in our center. This analysis showed that in haploidentical transplantation for AML, HLA-C group 1 mismatching in the graft vs host direction not only provides a survival advantage over non-NK-alloreactive (KIR ligand-matched) transplants (5-year EFS 67+/-10% vs 17+/-5%) but, indeed, also provides the best EFS compared with C2 (35+/-10%) or Bw4 KIR ligand mismatches (44+/-17%). In conclusion, we show that the kinetics with which single KIR-expressing NK cells are generated after haploidentical SCT differ between individual KIR receptors and seem to influence survival after haploidentical SCT.Bone Marrow Transplantation advance online publication, 22 February 2010; doi:10.1038/bmt.2010.19.

Genes Immun. 2010 Mar 4. [Epub ahead of print]

Signatures of natural selection and coevolution between killer cell immunoglobulin-like receptors (KIR) and HLA class I genes.

Guinan KJ, Cunningham RT, Meenagh A, Gonzalez A, Dring MM, McGuinness BW, Middleton D, Gardiner CM.
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Natural killer (NK) cells are lymphocytes of the innate immune system. In humans, NK cell activities are partly controlled by the diverse killer immunoglobulin-like receptor (KIR) gene family. The importance of NK cells in both immunity to infection and reproduction makes KIR strong candidates for genes undergoing dynamic evolution in the human genome. Using high-resolution allelic typing, we investigated the potential role of natural selection in the diversification of KIR in the Irish population. Higher diversity than expected is observed at several loci, consistent with a history of balancing selection acting to maintain several allelic variants at high frequency in the population. KIR diversity is enhanced further at the haplotype level with functional polymorphisms at KIR2DL4, KIR3DL1 and KIR2DS4 defining nine 'core' haplotypes. Analysis of these core haplotypes in combination with human leukocyte antigen (HLA) class I ligands revealed several nonrandom associations. In particular, the KIR:HLA association for the core haplotype defined by KIR3DL1(*)01502 was female specific and a likely consequence of negative selection acting against KIR3DL1(*)01502 on an HLA-C1/C1 background. Many of the associations between KIR and HLA in the Irish differ from those previously reported, which argues against universal selective pressures for specific KIR:HLA combinations in diverse human populations.

Proc Natl Acad Sci U S A. 2010 Mar 8. [Epub ahead of print]

Membrane nanotubes facilitate long-distance interactions between natural killer cells and target cells.

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Division of Cell and Molecular Biology, Imperial College London, London SW7 2AZ, United Kingdom.

Membrane nanotubes are membranous tethers that physically link cell bodies over long distances. Here, we present evidence that nanotubes allow human natural killer (NK) cells to interact functionally with target cells over long distances. Nanotubes were formed when NK cells contacted target cells and moved apart. The frequency of nanotube formation was dependent on the number of receptor/ligand interactions and increased on NK cell activation. Most importantly, NK cell nanotubes contained a submicron scale junction where proteins accumulated, including DAP10, the signaling adaptor that associates with the activating receptor NKG2D, and MHC class I chain-related protein A (MICA), a cognate ligand for NKG2D, as occurs at close intercellular synapses between NK cells and target cells. Quantitative live-cell fluorescence imaging suggested that MICA accumulated at small nanotube synapses in sufficient numbers to trigger cell activation. In addition, tyrosine-phosphorylated proteins and Vav-1 accumulated at such junctions. Functionally, nanotubes could aid the lysis of distant target cells either directly or by moving target cells along the nanotube path into close contact for lysis via a conventional immune synapse. Target cells moving along the nanotube path were commonly polarized such that their uropods faced the direction of movement. This is the opposite polarization than for normal cell migration, implying that nanotubes can specifically drive target cell movement. Finally, target cells that remained connected to an NK cell by a nanotube were frequently lysed, whereas removing the nanotube using a micromanipulator reduced lysis of these target cells.