Adaptor proteins contribute to the selection, differentiation and activation of natural killer T (NKT) cells, an innate-like lymphocyte population endowed with powerful immunomodulatory properties. Distinct from conventional T lymphocytes NKT cells preferentially home to the liver, undergo a thymic maturation and differentiation process and recognize glycolipid antigens presented by the MHC class I-like molecule CD1d on antigen presenting cells. NKT cells express a semi-invariant T cell receptor (TCR), which combines the Va14-Jα18 chain with a VB2, VB7, or VB8 chain in mice and the Va24 chain with the VB11 chain in humans. The avidity of interactions between their TCR, the presented glycolipid antigen and CD1d govern the selection and differentiation of NKT cells. Compared to TCR ligation on conventional T cells engagement of the NKT cell TCR delivers substantially stronger signals, which trigger the unique NKT cell developmental program. Furthermore, NKT cells express a panoply of primarily inhibitory NK cell receptors (NKRs) that control their self-reactivity and avoid autoimmune activation. Adaptor proteins influence NKT cell biology through the integration of TCR, NKR and/or SLAM (signaling lymphocyte-activation molecule) receptor signals or the variation of CD1d-restricted antigen presentation. TCR and NKR ligation engage the SH2 domain-containing leukocyte protein of 76kDa slp-76 whereas the SLAM associated protein SAP serves as adaptor for the SLAM receptor family. Indeed, the selection and differentiation of NKT cells selectively requires co-stimulation via SLAM receptors. Furthermore, SAP deficiency causes X-linked lymphoproliferative disease with multiple immune defects including a lack of circulating NKT cells. While a deletion of slp-76 leads to a complete loss of all peripheral T cell populations, mutations in the SH2 domain of slp-76 selectively affect NKT cell biology. Furthermore, adaptor proteins influence the expression and trafficking of CD1d in antigen presenting cells and subsequently selection and activation of NKT cells. Adaptor protein complex 3 (AP-3), for example, is required for the efficient presentation of glycolipid antigens which require internalization and processing. Thus, our review will focus on the complex contribution of adaptor proteins to the delivery of TCR, NKR and SLAM receptor signals in the unique biology of NKT cells and CD1d-restricted antigen presentation.

Keywords: NKT cells, CD1d, adaptor proteins, T cell receptor, NK cell receptor, differentiation, polarization
INTRODUCTION

Specific and appropriate intercellular interactions or the communication of cells with their environment requires the integration and coordination of multiple signaling pathways. Adaptor proteins contain a series of protein-binding sites that link respective interaction partners to each other and facilitate the generation of larger signaling complexes (1). This is, for example, pivotal for the delivery of signals from the T cell receptor (TCR) which plays a critical role in T cell biology (2).

There exist several T cell populations with distinct functions (3). Alpha beta (αβ) T cells, for example, termed conventional (αβ) T cells, are predominantly part of the adaptive immune system and display a large TCR diversity. TCR ligation by self-peptides embedded in major histocompatibility complex (MHC) molecules on antigen-presenting cells (APCs) in the thymus determines the fate of developing conventional T cells. Weak TCR signals perpetuate positive selection whereas strong, agonist, signals support the removal of potentially self-reactive cells acquire memory cell features during thymic maturation (4). Positive selection is an early step of TCR signaling events that contribute to the maturation, expansion and differentiation processes, termed stages 1–3 (Figure 1). These include the induction and regulation of promyelocytic leukemia zinc finger PLZF, the iNKT cell lineage transcription factor, multiple rounds of intrathymic cell divisions, the acquisition of a memory phenotype, the activation of cytokine gene loci, and the expression of multiple NKRs over time (5).

As NKR signaling engages also adaptor proteins, the propagation of signal transduction through adaptor molecules is in particular critical for diverse ranges of cellular processes in iNKT cells.

In contrast to conventional T cells, iNKT cells respond to glycolipid antigens and home predominantly to the liver (12). Unlike the development of conventional T cell, the selection of iNKT cells requires antigen presentation by double-positive thymocytes rather than thymic epithelial cells (13–17). iNKT cells are selected on high-affinity self-glycolipid ligands presented by the MHC class I-like molecule CD1d (18) which triggers their unique developmental program (19). Their selection uniquely requires co-stimulation via SLAM (signaling lymphocyte-activation molecule) family members and the tyrosine kinase Fyn (20–32) as discussed below. Once selected in stage 0, iNKT cells pass through complex activation, expansion, maturation and differentiation processes, termed stages 1–3 (Figure 1). These include the induction and regulation of promyelocytic leukemia zinc finger PLZF, the iNKT cell lineage transcription factor, multiple rounds of intrathymic cell divisions, the acquisition of a memory phenotype, the activation of cytokine gene loci, and the expression of multiple NKRs over the course of several weeks (7, 33). Although associated with their development (33, 34), PLZF is not unique to iNKT cells and also expressed in innate lymphoid cells (ILCs), mucosa-associated semi-invariant T (MAIT) cells and subsets of γδ T cells (35–37).

Furthermore, iNKT cells differentiate into three polarized subsets, NKT1, NKT2, and NKT17 cells (38) before egress into the periphery (Figure 1). Although TCR signal strength has been implicated in the polarization of the three iNKT cell sublineages and the regulation of PLZF expression (39), the intrathymic branching traits and cellular and molecular mechanisms of sublineage diversification are still under investigation. TCR-specific signals contribute also to the tissue distribution and phenotypic presentation of iNKT cells (40, 41). Although the signal delivered through the iNKT cell TCR is stronger than for the conventional T cell TCR (6, 42–44), the role of the TCR signal strength in iNKT cell lineage commitment and differentiation is still under investigation.

Next to αβ-TCR+ iNKT cells CD1d-restricted γδ T cells also respond to (glycol)-lipid antigens (45). These γδ T cells

NATURAL KILLER T (NKT) CELLS AND CD1D-MEDIATED ANTEN

Natural killer T (NKT) cells belong to the group of innate(-like) unconventional T cells. They explosively release various cytokines and chemokines upon TCR engagement and thus, exhibit powerful immunomodulatory properties. NKT cells can be divided into two distinct lineages, namely type 1 or invariant NKT cells and type 2 NKT cells. Type 2 NKT cells exhibit a more diverse TCR repertoire. In contrast, type 1 or invariant NKT cells—hereinafter referred to as iNKT cells—express a semi-invariant canonical T cell receptor (TCR), which combines the Vα14-Jα18 chain with the Vβ2, Vbeta7, or Vbeta8 chain in mice and the Vα24-Jα18 chain with the Vβ11 chain in humans. Simultaneously, they carry a wide range of activating and inhibitory NK cell receptors (NKR)s on their surface (7). The inhibitory NKR$s presumably control the self-reactivity of iNKT cells and avoid autoimmune activation (8, 9). Vice versa, the NKT cell TCR shapes the pattern of NKR expression, as exemplified for Ly49 receptors (10). Furthermore, balanced signaling through activating and inhibitory NKR$s might influence the developmental program of iNKT cells (11). As NKR signaling engages also adaptor proteins, the propagation of signal transduction through adaptor molecules is in particular critical for diverse ranges of cellular processes in iNKT cells.
express γδ1 and δ6.3 chains and the promyelocytic leukemia zinc finger (PLZF), the lineage transcription factor of NKT cells. Further comparisons of γδ+ with αβ-TCR expressing NKT cells revealed also converging patterns of cytokine, gene and cell surface marker expression implying similar differentiation programs in both NKT cell subsets (33, 34, 37, 46–48). Thus, several observations obtained with αβ-TCR+ iNKT cells, might be reflected in the biology of CD1d-restricted γδ T cells.

Another feature of iNKT cells distinct from conventional T cells is the recognition of glycolipid antigens presented by CD1d. CD1d molecules are assembled in the endoplasmatic reticulum (ER) as non-covalently linked heterodimers of an isotype-specific heavy chain and β-2-microglobulin (β2m). During its assembly in the ER, CD1d incorporates endogenous lipids and traffics to the plasma membrane. While certain lipids can load onto CD1d directly at the cell surface, CD1d with its hydrophobic binding groove of intermediate size usually has to recycle into late endosomal and lysosomal compartments for efficient antigen exchange and loading (49, 50). Upon trafficking back to the cell surface, antigens are presented by CD1d to NKT cells (51, 52).

ADAPTOR PROTEINS IN INKT CELL BIOLOGY

Adaptor molecules are multi-domain proteins lacking intrinsic catalytic activity, functioning instead by nucleating molecular complexes during signal transduction (53). Several adaptor proteins influence iNKT cell selection, differentiation and activation, either intrinsically or indirectly through interference with CD1d-mediated antigen presentation. For example, one of the pivotal molecules engaged upon TCR ligation is the intracellular adaptor protein slp-76. While the complete absence of slp-76 (54–56) or of its N-terminal region (57) leads to a lack of all peripheral T cell populations, selective mutations in the SH2 domain of slp-76 affect in particular iNKT cells (58). Most importantly and in strict contrast to conventional T cells, the selection of iNKT cells requires co-stimulation via SLAM (signaling lymphocyte-activation molecule) family members (20–24). Thus, the SLAM-associated adaptor protein (SAP) signaling pathway is selectively required for iNKT cell development. Adaptor proteins, however, can also influence CD1d expression by antigen presenting cells (APCs) and subsequently affect iNKT cell biology in an extrinsic manner. Adaptor protein complex 3 (AP-3), for example, is required for the efficient presentation of glycolipid antigens that require internalization and processing (59).

The slp-76 Family of Adaptor Proteins

The slp-76 family of adapters includes the SH2 domain-containing leukocyte phosphoprotein of 76 kDa (slp-76), the B cell linker protein (BLNK), and the cytokine-dependent hematopoietic cell linker (CLNK) (53). All three proteins interact with similar but not identical signaling molecules and are critical for the integration of multitudinous signal cascades downstream of immunotyrosine-based activation motif (ITAM)-bearing receptors and integrins in various hematopoietic cell populations (60). Slp-76 is expressed in T cells, monocytes/macrophages, NK cells, mast cells and platelets (61, 62). BLNK reflects the slp-76 homolog in B cells. It shares about a 33% amino acid identity, but some of its structural domains are similar to those of slp-76 (60, 63, 64). BLNK is primarily responsible for the transmission of signals through the B cell receptor (BCR). CLNK is selectively expressed in various hematopoietic cells following cytokine stimulation (65).

The SH2 Domain-Containing Leukocyte Phosphoprotein of 76 kDa, Slp-76

Of these three family members primarily slp-76 is pivotal for T cell development and TCR signaling (61, 62). Due to impaired signals from the pre-TCR, double negative 3 (DN3) T cells
cannot transform into the double negative 4 (DN4) stage (54, 55, 57). Consequently, slp-76^{-/-} mice lack all peripheral mature T cells (57).

The divergent functions of slp-76 are mediated by its distinct signaling domains (Figure 2). The N-terminal acidic domain contains three tyrosine residues (66) which become phosphorylated by the protein tyrosine kinase Zap-70 upon TCR ligation (67, 68) and subsequently bind the SH2 domains of the guanine nucleotide exchange factor Vav (68–70), the adaptor protein Nck (71, 72) and the Tec-family kinase Itk (73, 74). The deletion of this N-terminal region (57) leads to a lack of all peripheral T cell populations, similar as the complete knockout of slp-76 protein (54, 55, 57). Of these three binding partners in particular Itk affects the development, maturation, cytokine production and survival of NKT cells (75–79). Itk-deficiency affected thereby not only α/β-TCR-, but also γ/δ-TCR-expressing NKT cells which in particular affect the control of Th2 responses and IgE production (80).

The central proline-rich domain of slp-76 interacts with the phospholipase PLCγ-1 (81) and the adaptor molecule GADS (Grb2-related adaptor downstream of Shc) (82). For none of these two molecules a role in NKT cell biology has been established so far.

The C-terminal SH2 domain of slp-76 binds to the serine-threonine kinase HPK-1 (hematopoietic progenitor kinase 1) (83) and to the adhesion and degranulation-promoting adaptor protein (ADAP) (84, 85). ADAP is required for thymocyte selection and TCR-mediated integrin activation (86–88). Thus, slp-76 interferes with inside-out and outside-in signaling cascades and integrin-expression (89) due to its multipoint binding with ADAP (90).

A missense mutation within the SH2-domain of slp-76 led to an accumulation of iNKT cells in the thymus and in peripheral lymph nodes. In contrast, iNKT cells were selectively reduced in the spleens and livers of mice with the same mutation, along with a reduced cytokine response, decreased levels of ADAP protein and altered integrin and NKR expression patterns (58). Although TCR signals were affected by these mutations, NKR expression in mice with this slp-76 mutation for iNKT cells needs to be characterized in further detail by assessing the alterations in subsequent signaling pathways and by screening additional slp-76 mutations. Interestingly, despite exhibiting an NKR distribution that has been associated with enhanced Th1 polarization (7, 38), a simultaneous reduction of both IL-4- and IFN-γ-expression along with a reduced TCR-reactivity was observed in iNKT cells carrying this missense mutation within the SH2-domain of slp-76 (58). Thus, variations in the tissue distribution rather than the cytokine polarization are to be considered in patients with allelic mutations in TCR signaling molecules before pursuing vaccination strategies involving α-GalCer, the prototypical iNKT cell ligand as an adjuvant.

The Cytokine-Dependent Hematopoietic Cell Linker (clnk)

Next to cytokine driven expression clnk plays a role in Fc-epsilon R1-mediated mast cell degranulation, B cell receptor (BCR) and TCR signaling (60, 65). While not found in resting T cells, clnk is abundantly expressed in previously activated T cells (65). Similar to slp-76, clnk consists of a tyrosine- and proline-rich amino-terminal basic domain, an SH2 domain and a carboxy-terminal tail (60). While the SH2 domains of slp-76 and clnk exhibit the highest degree of homology within their SH2 domains the sequence variations outside this region suggest that clnk might not be phosphorylated by Zap-70 and does not associate with Vav, Nck, or GADS. Clnk can rescue TCR signals in slp-76-deficient T cells (65), but clnk itself is dispensable for T cell function and differentiation (93). Clnk might contribute to the coordination of antigen-receptor signaling and cytokine stimulation. Interestingly, clnk might mediate diverse or even opposite signals by TCRs and NRks as it promotes iNKT cell responses, but impairs NK cell function (94). Thus, clnk might function as a molecular switch, which controls diverse immune responses in different cell populations.

Signaling Lymphocytic Activation Molecule (SLAM) and Signaling Lymphocytic Activation Molecule-Associated Protein (SAP)

The signaling lymphocytic activation molecule (SLAM) family of cell surface receptors comprises six members named 2B4 (CD244), Ly9 (CD229), CRACC SLAM (CD150), CD84, and Ly108 (95, 96) which are exclusively expressed on hematopoietic cells. They represent homophilic receptors with the exception of 2B4, which recognizes CD48. SLAM family receptors possess an extracellular segment with two or four immunoglobulin-like domains responsible for ligand recognition, a single transmembrane region and a cytoplasmic domain. This cytoplasmic domain bears one to three inhibitory or activating immunoreceptor tyrosine-based switch motifs (ITSMs) (97).

Signaling lymphocytic activation molecule (SLAM)-associated proteins (SAPs) are adaptor molecules which contain Src homology 2 (SH2) domains. SAPs are expressed in T cells, NK cells, and iNKT cells. The SAP family of adaptors includes three members most commonly known as SAP (also named SH2D1A), Ewing’s sarcoma-associated transcript-2 (EAT-2; also named SH2D1B1) and EAT-2-related transducer (ERT; also named SH2D1B2) (98). Mutations in the SAP (SH2D1A) gene located on chromosome X are responsible for X-linked lymphoproliferative disease (XLP), characterized by higher susceptibility to Epstein-Barr virus (EBV) infection, B cell lymphomas, severe immune dysregulation, a nearly complete loss of iNKT cells and an impaired humoral immunity (22, 23, 99–102). The correlation of an augmented susceptibility to EBV infections with the lack of
iNKT cells together with the observation that the SLAM family receptor 2B4 exhibits defect signaling function in SAP-deficiency (103–105) suggest a key role for iNKT cells and SLAM family receptors in the immune response to EBV.

SAP family adaptor proteins respond through their SH2 domains to the cytoplasmic domains of SLAM family receptors by recruiting and activating the downstream tyrosine kinase Fyn (Figure 3) (106). However, SLAM family receptors can also signal through other SH2 domain-containing molecules such as the protein tyrosine phosphatases SHP-1 and SHP-2 or the SH2 domain inositol phosphatase 1 (SHIP-1) and SHIP-2, particularly in SAP deficiency (25, 97, 101, 107–111). While SAP-dependent SLAM family receptor signaling is pivotal for the selection of iNKT cells, these receptors inhibit SAP-independently follicular helper T cells and humoral immune responses (25).

iNKT cells are known to use unique signaling pathways (26). Fyn, for example, is required for iNKT cell development, but not for the differentiation of conventional T lymphocytes or NK cells (20, 21). The loss of SAP resulted in a complete absence of iNKT cells from both mice and humans. SAP-transmitted signaling events were uniquely required for the development of iNKT cells, as conventional T cells and NK cells developed normally in the absence of SAP (22, 23). The selection of iNKT cells also strictly requires co-stimulation via SLAM (signaling lymphocyte-activation molecule) family members (20–24). Homotypic interactions involving the SLAM family receptors 1 and 6 are required for iNKT cell differentiation (24). While SAP deficiency blocks positive selection at stage 0, the most immature stage of iNKT cell development (22, 23), mice lacking SLAM receptors exhibit less pronounced iNKT cell defects that appear to spare stage 0 iNKT cells (24, 25). Indeed, unlike SAP, SLAM family receptors promoted iNKT cell development and intrathymic maturation due to the restriction of TCR signal strength following positive selection and the limitation of activation induced cell death (27). This process involves the adaptor SAP-kinase Fyn complex and the protein tyrosine phosphatases SHP-1. Thus, this study uncovers important differences in SAP and SLAM signaling and highlights the complex processes underlying iNKT cell maturation and survival (112) as auto-reactive iNKT cell activation during thymic selection is thought to induce a substantially stronger TCR stimulus in comparison to that during the development of conventional T cells (6, 113). As a consequence the expression of the transcription factors Egr1 and Egr2 is strongly increased (113), which in turn directly induce PLZF, the key transcription factor controlling iNKT cell differentiation, migration, and functions (113). SAP regulates also cytokine production, expression of transcription factors, the polarization of iNKT cells favoring the development of NKT2 cells and the formation of the immunologic synapse (28, 114, 115). Furthermore, SAP expression in iNKT cells promotes cognate help to B cells (116, 117). Thus, the SLAM-associated adaptor protein (SAP) signaling pathway is selectively required for iNKT cell development and the loss of iNKT cells has been suggested.
to contribute to the genesis of the lethal immunodeficiency syndrome. The need for SAP-mediated signals may reflect the unique requirements for the positive selection of iNKT cells in the thymus. However, several questions remain unresolved. For example, the role of individual SLAM family receptors in cytokine polarization and iNKT cell differentiation needs to be characterized in more detail as well as the impact of subsequent signaling cascades and their interference with NKR and TCRs. In addition, it is still unknown, whether and how TCR and SLAM family receptors interfere on a cellular and molecular level and why this is specific for iNKT cells.

Adaptor Protein-3 (AP-3)

The hetero-tetrameric AP (adaptor protein) complexes are involved in the sorting of cargo proteins into transport vesicles that traffic between the different organelles of the cell. They are known to bind to the tyrosine or dileucine-containing sequence motifs in transmembrane proteins in order to direct their selective localization to subsets of endosomal and lysosomal compartments (118, 119). Five members, AP-1 to AP-5 and their isoforms have been characterized in this family of cytosolic complexes (118–120). In contrast to AP-4 and-5, AP-1,-2, and-3 are clathrin-associated complexes (121). AP-1 and AP-2 direct proteins from the trans-Golgi network to endosomes and recycling compartments, respectively (122, 123). AP-3 localizes membrane proteins to lysosomes, platelet-dense granules, and melanosomes (124). AP-3-deficient mice as well as Hermansky-Pudlak syndrome type 2 (HPS-2) patients with mutations in the AP-3 gene exhibited hypopigmentation and platelet dysfunction (125–129). AP-4 mediates vesicle trafficking from the trans-Golgi network to endosomes or the basolateral plasma membrane. The function of AP-5 localized in late endosomes is largely unknown (121). To date, there have been no interactions between AP-1, AP-4, and AP-5 with CD1d described. However, CD1d directly interacts with AP-2, which targets the endosomal compartment, and AP-3, which targets the lysosomal compartment (59, 130). Indeed, AP-2 restrains iNKT cell activation due to the regulation of CD1d internalization (131), and a connection of AP-2 with autophagy as a regulator of iNKT cell activation, development and survival is currently under investigation. In this context, a deletion of the essential autophagy gene Atg7 abrogated thymic iNKT cell development and peripheral iNKT cell functions in a cell-intrinsic manner (132, 133). Unexpectedly, however, Atg7-deficient thymocytes and bone marrow-derived DCs exhibited no defect in the presentation of glycolipid antigens, implying distinct differences in the mechanisms how AP-2 and autophagy genes affect iNKT cell development and activation that need to be dissected in the future.

In contrast, numerous studies have investigated the interaction of AP-3 and CD1d. Since CD1d recycles between the cell membrane and the lysosome back and forth, AP-3 interferes with glycolipid metabolism and CD1d-mediated (glyco-)lipid antigen presentation (134). Indeed, it was shown that AP-3 is required for the efficient presentation of glycolipid antigens that require internalization and processing (59, 135). AP-3 interacts with CD1d, but does not affect MHC II presentation (59, 135–137). Cells from AP-3-deficient mice show increased cell surface expression of CD1d but decreased expression in late endosomes. Consequently, AP-3-deficient splenocytes present glycolipids to iNKT cells less efficiently. Furthermore, AP-3-deficient mice exhibit significantly reduced iNKT cell numbers. The simultaneous analysis of CD1d mutants with alterations in the cytoplasmic tail to AP-3-knockout mice proved also that CD1d molecules in lysosomes are functional in antigen presentation (59, 130). iNKT cell numbers are reduced in patients with Hermansky-Pudlak syndrome type 2 (HPS-2) (138) and iNKT cell defects have been also associated with the susceptibility to infections and lymphoma in patients with this homozygous genomic AP-3 deletion (139). Thus, in summary these studies showed that the localization of CD1d to late endosomes or lysosomes is required for both (glycolipid antigen presentation and the subsequent development of iNKT cells. These reports also demonstrated that different pathways mediate the intracellular trafficking of MHC II and CD1 molecules, which both scavenge late endosomes or lysosomes.

**FIGURE 3** SLAM receptor signaling. SLAM receptor ligation recruits and activates via SAP the protein tyrosine kinase FYN, which phosphorylates SLAM and subsequently generates docking sites for SRC homology 2 (SH2)-domain-containing downstream adaptor proteins and enzymes. SLAM engagement also cooperates with TCR-mediated signals leading to nuclear factor-κB (NF-κB) activation, cytokine production and NKT cell development. DOK1 and 2, docking proteins 1 and 2; RasGAP, RAS-GTPase-activating protein; SAP, Signaling lymphocytic activation molecule (SLAM)-associated proteins; SLAM, signaling lymphocytic activation molecule; SHIP, SH2-domain-containing inositol–5’-phosphatase.
CONCLUSION

Adaptor proteins play a pivotal role in the biology of CD1d-restricted iNKT cells. SAP transfers SLAM receptor signals, propagates the thymic selection of iNKT cells and induces the iNKT cell effector program (33). The SH2 domain of slp-76 influences the tissue distribution and phenotype of iNKT cells in the periphery (58). AP-3 interferes with the presentation of glycolipid antigens by CD1d (59). Thus, these three adaptor proteins engage unique functions in iNKT cells biology distinct from conventional T lymphocytes. Particularly the expression of SAP and slp-76 in iNKT cells raises the question whether these two molecules interact (Figure 4). As SLAM receptors, NKR and TCRs share adaptor proteins for signal transmission (140, 141), it will be interesting to define the contribution of the respective receptors to the observed phenotypes. Another interesting candidate to investigate in this context is the protein tyrosine kinase SHP-1 since it also interferes with all three receptor classes (111, 116, 142–144) and localizes with slp-76 and fyn in lipid rafts (145–147), even though evidence of physical interactions of these three molecules in iNKT cells is missing. As the strength of the TCR signals influences the polarization of iNKT cell subsets (39), the role of adaptor proteins in fine-tuning intracellular signal transduction is to characterize. In addition, as SLAM receptors are pivotal for the induction of the iNKT cell lineage transcription factor PLZF (33) and PLZF expression negatively correlates with the glycolytic potential of iNKT cells (148) potential connections between adaptor proteins and iNKT cell metabolism need to be identified.

AUTHOR CONTRIBUTIONS

EG prepared the figures and added comments to the manuscript. JM wrote the manuscript.

FUNDING

This study was supported by the Staedtler Stiftung (to JM), the German Research Foundation DFG (grant MA 2621/4-1 to JM and DFG-CRC1181—project number C04 to JM).

ACKNOWLEDGMENTS

We thank all the present members of our lab for their support and their contributions.

REFERENCES

1. Flynn DC. Adaptor proteins. Oncogene. (2001) 20:6270–2. doi: 10.1038/sj.onc.1204769
2. Peterson EJ, Clements JL, Fang N, Koretzky GA. Adaptor proteins in lymphocyte antigen-receptor signaling. Curr Opin Immunol. (1998) 10:337–44. doi: 10.1016/S0952-7915(98)80173-8
3. Edholm ES, Banach M, Robert J. Evolution of innate-like T cells and their selection by MHC class I-like molecules. Immunogenetics. (2016) 68:525–36. doi: 10.1007/s00251-016-0929-7
4. Moran AE, Hogquist KA. T-cell receptor affinity in thymic development. Immunology. (2012) 135:261–7. doi: 10.1111/j.1365-2567.2011.03547.x
5. Legoux F, Salou M, Lantz O. Unconventional or preset alphabeta T cells: evolutionarily conserved tissue-resident T cells recognizing nonpeptidic ligands. Annu Rev Cell Dev Biol. (2017) 33:511–35. doi: 10.1146/annurev-cellbio-100616-060725
6. Moran AE, Holzapfel KL, Xing Y, Cunningham NR, Maltzman JS, Punt J, et al. T cell receptor signal strength in Treg and iNKT cell development demonstrated by a novel fluorescent reporter mouse. J Exp Med. (2011) 208:1279–89. doi: 10.1084/jem.20110308
7. Bendelac A, Savage PB, Seyton L. The biology of iNKT cells. Annu Rev Immunol. (2007) 25:297–336. doi: 10.1146/annurev.immunol.25.022106.141711
8. Bendelac A, Bonneville M, Kearney JF. Autoreactivity by design: innate B and T lymphocytes. Nat Rev Immunol. (2001) 1:177–86. doi: 10.1038/35105025
9. Kronenberg M, Rudensky A. Regulation of immunity by self-reactive T cells. Nature. (2005) 435:598–604. doi: 10.1038/nature03725
30. Huang B, Gomez-Rodriguez J, Preite S, Garrett LJ, Harper UL, Schwartzberg PL. CRISPR-mediated triple knockout of SLAMF1, SLAMFS1 and SLAMFS6 supports positive signaling roles in NKT cell development. PLoS ONE. (2016) 11:e0156072. doi: 10.1371/journal.pone.0156072

31. Baglaenko Y, Cruz Teugalubova M, Gracey E, Talaei N, Manion KP, Chang NH, et al. Invariant NKT cell activation is potentiated by homotypic trans-ly40 interactions. J Immunol. (2017) 198:3949–62. doi: 10.4049/jimmunol.1601369

32. Cuencà M, Punet-Ortiz J, Raulet D, Terhorst C, Engel P, Ly9 (SLAMF3) receptor differentially regulates iNKT cell development and activation in mice. Eur J Immunol. (2018) 48:99–105. doi: 10.1007/ej.201746925

33. Savage AK, Constantines MG, Han J, Picard D, Martin E, Li B, et al. The transcription factor PLZF directs the effector program of the NKT cell lineage. Immunity. (2008) 29:391–403. doi: 10.1016/j.immuni.2008.07.011

34. Kovalovsky D, Uche OU, Eladad S, Hobbs RM, Yi W, Alonzo, E et al. The B73-zinc finger transcriptional regulator PLZF controls the development of invariant natural killer T cell effector fucntions. Nat Immunol. (2008) 9:1055–64. doi: 10.1038/nj.16141

35. Constantines MG, McDonald BD, Verhoeef PA, Bendelac A. A committed precursor to innate lymphoid cells. Nature. (2014) 508:397–401. doi: 10.1038/nature13047

36. Koay HE, Gherrardin NA, Enders A, Loh L, Mackay LK, Almidae CF, et al. A three-stage intrathyamic development pathway for the mucosal-associated invariant T cell lineage. Nat Immunol. (2016) 17:1300–11. doi: 10.1038/nj.2015.365

37. Kreslavsky T, Savage AK, Hobbs R, Gounari F, Bronson R, Pereira P, et al. TCR-inducible PLZF transcription factor required for innate phenotype of a subset of gammadelta T cells with restricted TCR diversity. Proc Natl Acad Sci USA. (2009) 106:12453–8. doi: 10.1073/pnas.0903895106

38. Lee YJ, Holzapfel KL, Zhu J, Jameson SC, Hogquist KA. Steady-state production of IL-4 modulates immunity in mouse strains and is determined by lineage diversity of iNKT cells. Nat Immunol. (2013) 14:1146–54. doi: 10.1038/nj.2013.2731

39. Tuttle KD, Krovi SH, Zhang J, Bedel R, Harmackle L, Peterson, K, et al. TCR signal strength controls thymic differentiation of iNKT cell subsets. Nat Commun. (2018) 9:2650. doi: 10.1038/s41467-018-05026-6

40. Subleski JJ, Hall VL, Wolfe TB, Scarzello M, Weiss JM, Chan T, et al. TCR-dependent and -independent activation underlie liver-specific regulation of NKT cell subsets. J Immunol. (2011) 186:838–47. doi: 10.4049/jimmunol.1001735

41. Lee YJ, Wang H, Starrett GL, Phuong V, Jameson SC, Hogquist KA. Tissue-specific distribution of iNKT cells impacts their cytokine response. Immunity. (2015) 43:566–78. doi: 10.1016/j.immuni.2015.06.025

42. Stritesky GL, Jameson SC, Hogquist KA. Selection of self-reactive T cells in the thymus. Annu Rev Immunol. (2012) 30:95–114. doi: 10.1146/annurev-immunol-020711-075035

43. Hogquist KA, Jameson SC. The self-obsession of T cells:how TCR signaling thresholds affect fate decisions and effector function. Nat Immunol. (2014) 15:815–23. doi: 10.1038/nij.2013.293

44. Gapon L. Development of invariant natural killer T cells. Curr Opin Immunol. (2016) 49:99–105. doi: 10.1016/j.coi.2016.01.001

45. Luoma AM, Castro CD, Adams EJ, gammadelta T cell surveillance via CD1 molecules. Trends Immunol. (2014) 35:613–21. doi: 10.1016/j.ti.2014.09.003

46. Alonso ES, Gottschalk RA, Das J, Egawa T, Hobbs RM, Pandolfi, PP, et al. Development of promyeolocytic zinc finger and ThPOK-expressing innate gamma delta T cells is controlled by strength of TCR signaling and Id3. J Immunol. (2010) 184:1268–79. doi: 10.4049/jimmunol.0903218

47. Verykokakis M, Boos MD, Bendelac A, Adams EJ, Pereira P, Kee BL. Inhibitor of DNA binding 3 limits development of murine slm-associated adaptor protein-dependent ‘innate’ gammadelta T cells. PLoS ONE. (2010) 5:e9303. doi: 10.1371/journal.pone.0009303

48. Narayan K, Sylvia KE, Malhotra N, Yin CC, Martens G, Vallerskog T, et al. The crystal structure of human CD1d with alpha-galactosylceramide. Nat Immunol. (2005) 6:819–26. doi: 10.1038/nj.2005.1225

49. Zajonc DM, Cantu C III, Mattner J, Zhou D, Savage PB, Bendelac A, et al. Structure and function of a potent agonist for the semi-invariant natural killer T cell receptor. Nat Immunol. (2005) 6:810–8. doi: 10.1038/nj.2005.1224
51. Hava, DL, Brigit M, van den Elzen P, Zajonc DM, Wilson IA, Brenner MB. CD1 assembly and the formation of CD1-antigen complexes. *Curr Opin Immunol.* (2005) 17:88–94. doi: 10.1016/j.coi.2004.12.003

52. Girardi E, Zajonc DM. Molecular basis of lipid antigen presentation by CD1d and recognition by natural killer T cells. *Immunol. Rev.* (2012) 250:167–79. doi: 10.1111/j.1600-065X.2012.01166.x

53. Wu JN, Koretzky GA. The SLP-76 family of adaptor proteins. *Semin Immunol.* (2004) 16:379–93. doi: 10.1016/j.smim.2004.08.018

54. Clements JL, Ross-Barta SE, Tygrett LT, Waldschmidt TJ, Koretzky GA. SLP-76 in antigen-stimulated T cells. *Science.* (1998) 281:416–9. doi: 10.1126/science.281.5375.416

55. Pivniouk V, Tsetisou E, Swinton P, Rathbun G, Geha RS. Impaired viability and profound block in thymocyte development in mice lacking the adaptor protein SLP-76. *J Exp Med.* (1998) 194:229–38. doi: 10.1084/jem.194.6.867

56. Myung PS, Boerthe NJ, Koretzky GA. Adapter proteins in lymphocyte antigen-receptor signaling. *Curr Opin Immunol.* (2000) 12:256–66. doi: 10.1016/S0952-7915(00)00858-6

57. Jordan MS, Smith JE, Burns JC, Austin JE, Nichols KE, Aschbrenner AC, et al. Molecular cloning of SLP-76, a 76-kDa tyrosine phosphoprotein associated with Grb2 in T cells. *J Biol Chem.* (1995) 270:7029–32. doi: 10.1074/jbc.270.7029

58. Jackman JK, Motto DG, Sun Q, Tanemoto M, Turkc CW, Peltz GA, et al. Molecular cloning of SLP-76, a 76-kDa tyrosine phosphoprotein associated with Grb2 in T cells. *J Biol Chem.* (1995) 270:7029–32. doi: 10.1074/jbc.270.7029

59. Clements JL, Ross-Barta SE, Tygrett LT, Waldschmidt TJ, Koretzky GA. SLP-76 expression is restricted to hematopoietic cells of monocyte, granulocyte, and T lymphocyte lineage and is regulated during T cell maturation and activation. *J Immunol.* (1998) 161:3880–9.

60. Fu C, Turkc CW, Kurosaki T, Chan AC. BLNK: a central linker protein in B cell activation. *Immunity.* (1998) 9:93–103. doi: 10.1016/S1074-7613(00)00059-1

61. Goitsuka R, Fujimoto Y, Mamada H, Umeda A, Morimura T, Uetsuka K, et al. BASH, a novel signaling molecule preferentially expressed in B cells of the bursa of Fabricius. *J Immunol.* (1998) 161:5804–8.

62. Da Silva AJ, Li Z, de Vera C, Canto E, Findell P, Rudd CE. Cloning of a novel T cell protein FYB that binds FYN and SH2-domain-containing leukocyte protein 76 and modulates interleukin 2 production. *Proc Natl Acad Sci USA.* (2001) 98:12408–18. doi: 10.1073/pnas.21.13.4208-4218.2001

63. Felices M, Yin CC, Kosaka Y, Kang J, Berg LJ. The Tec kinase ITK regulates thymic emigration, and maturation and functionality of the NKT cells. *J Immunol.* (2003) 170:2659–69. doi: 10.4049/jimmunol.170.11.111

64. Wu JN, Koretzky GA, McCladek T, Weiss A. Identification of a phospholipase C-gamma1 (PLC-gamma1) SH3 domain-binding site in SLP-76 required for T-cell receptor-mediated activation of PLC-gamma1 and NFAT. *Mol Cell Biol.* (2001) 21:4208–18. doi: 10.1128/MCB.21.13.4208-4218.2001

65. Bubeck Wardenburg J, Pappu R, Bu JY, Mayer B, Chernoff J, Strauss D, et al. Regulation of PAK activation and the T cell cytoskeleton by the linker protein SLP-76. *Immunity.* (1998) 9:607–16. doi: 10.1016/S1074-7613(00)00856-5

66. Motto DG, Ross SE, Wu J, Hendricks-Taylor LR, Koretzky GA. Implication of ITK in survival of invariant NKT cells associated with the p53-dependent pathway in mice. *J Immunol.* (2012) 188:3611–9. doi: 10.4049/jimmunol.1102477

67. Wu JN, Gheith S, Bezman NA, Liu QH, Fostel LV, Swanson AM, et al. Molecular cloning of SLAP-130, an SLP-76-associated substrate of protein 76 and modulates interleukin 2 production. *Proc Natl Acad Sci USA.* (2006) 103:10522–7. doi: 10.1073/pnas.0511106103

68. Griffiths EK, Krawczyk C, Kong YY, Raab M, Hydock SJ, Bouchard D, et al. Positive regulation of T cell activation and integrin adhesion by the adapter Fyb/Slap. *Science.* (2001) 293:2260–3. doi: 10.1126/science.1063397

69. Peterson EL, Woods ML, Dmowski SA, Derimanov G, Jordan MS, Wu JN, et al. Coupling of the TCR to integrin activation by Slap-130. *Fibc.* (2001) 293:2263–5. doi: 10.1126/science.1063486

70. Griffiths EK, Penninger JM. Communication between the TCR and integrin: role of the molecular adapter ADAP/Fyb/Slap. *Curr Opin Immunol.* (2002) 14:317–22. doi: 10.1016/S0952-7915(02)00334-5
Gerth and Mattner
Adaptor Proteins and NKT Cells

109. Le Borgne M, Shaw AS. SAP signaling: a dual mechanism of action. *Immunity*. (2012) 36:899–901. doi: 10.1016/j.immuni.2012.06.002

110. Zhao F, Cannons JL, Dutta M, Griffiths GM, Schwartzberg PL. Positive and negative signaling through SLAM receptors regulate synapse organization and thresholds of cytolysis. *Immunity*. (2012) 36:1003–16. doi: 10.1016/j.immuni.2012.05.015

111. Wu N, Zhong MC, Roncagalli R, Perez-Quintero LA, Guo H, Zhang Z, et al. A hematopoietic cell-driven mechanism involving SLAMF6 receptor, SAP adaptors and SHP-1 phosphatase regulates NK cell education. *Nat Immunol*. (2016) 17:387–96. doi: 10.1038/ni.3369

112. Iyer SS, Huang YH, Blumberg RS. SLAM-ing the brakes on iNKT cell selection. *Nat Immunol*. (2019) 20:378–9. doi: 10.1038/s41590-019-0355-8

113. Seiler MP, Mathew R, Liszewski MK, Spooner CJ, Barr K, Meng F, et al. Elevated and sustained expression of the transcription factors Egr1 and Egr2 controls NKT lineage differentiation in response to TCR signaling. *Nat Immunol*. (2012) 13:264–71. doi: 10.1038/ni.2230

114. Cen O, Ueda A, Guzman L, Jain J, Bassiri H, Nichols KE, et al. The adaptor molecule signaling lymphocytic activation molecule-associated protein (SAP) regulates IFN-gamma and IL-4 production in V alpha 14 transgenic NKT cells via effects on GATA-3 and T-bet expression. *J Immunol*. (2009) 182:1370–8. doi: 10.4049/jimmunol.182.3.1370

115. Das R, Bassiri H, Guan P, Wiener S, Banerjee PP, Zhong MC, et al. The adaptor molecule SAP plays essential roles during invariant NKT cell cytotoxicity and lytic synapse formation. *Blood*. (2011) 123:3366–95. doi: 10.1182/blood-2011-11-468686

116. Kageyama R, Cannons JL, Zhao F, Yusuf I, Lao C, Locci M, et al. The receptor Lyt108 functions as a SAP adaptor-dependent on-off switch for T cell help to B cells and NKT cell development. *Immunity*. (2012) 36:986–1002. doi: 10.1016/j.immuni.2012.05.016

117. Detre C, Keszei M, Garrido-Mesa N, Kis-Toth K, Castro W, Agymeng AF, et al. SAP expression in invariant NKT cells is required for cognate help to support B-cell responses. *Blood*. (2012) 120:122–9. doi: 10.1182/blood-2012-11-395913

118. Boehm M, Bonifacino JS. Adapting: the final recount. *Mol Biol Cell*. (2001) 12:2907–20. doi: 10.1091/mbc.12.10.2907

119. Robinson MS, Bonifacino JS. Adaptor-related proteins. *Curr Opin Cell Biol*. (2001) 13:444–53. doi: 10.1016/S0959-4947(00)00235-0

120. Kirchhausen T. Adaptors for clathrin-mediated traffic. *Annu Rev Cell Dev Biol*. (1999) 15:705–32. doi: 10.1146/annurev.cellbio.15.1.705

121. Park SY, Guo X. Adaptor protein complexes and intracellular transport. *Biosci Rep*. (2014) 34:e00123. doi: 10.1042/BSR20140069

122. Huang F, Nesterov A, Carter RE, Sorokin A. Trafficking of yellow-fluorescent-protein-tagged mu subunit of clathrin adaptor AP-1 complex in living cells. *Traffic*. (2001) 2:343–57. doi: 10.1034/j.1600-0854.2001.2050206.x

123. Rapport I, Miyazaki M, Boll W, Duckworth B, Cantley LC, Sholson S, et al. Regulatory interactions in the recognition of endocytic sorting signals by AP-2 complexes. *EMBO J*. (1997) 16:2240–50. doi: 10.1093/emboj/16.9.2240

124. Daughtery BL, Straley KS, Sanders JM, Phillips JW, Didier M, McEvier RP, et al. AP-3 adaptor functions in targeting P-selectin to secretory granules in endothelial cells. *Traffic*. (2001) 2:406–13. doi: 10.1042/1525-888X:2001019

125. Kantheti P, Qiao X, Diaz ME, Peden AA, Meyer GE, Carskadon SL, et al. Mutation in AP-3 delta in the mocha mouse links endosomal transport to storage deficiency in platelets, melanosomes, and synaptic vesicles. *Neuron*. (1998) 21:111–22. doi: 10.1016/S0896-6273(00)80519-X

126. Dell’Angelica EC, Shotelersuk V, Aguilar RC, Gahl WA, Bonifacino JS. Altered trafficking of lysosomal proteins in Hermansky-Pudlak syndrome due to mutations in the beta 3A subunit of the AP-3 adaptor complex. *Hum Mol Genet*. (1999) 8:323–30. doi: 10.1093/hmg/8.2.323

127. Shotelersuk V, Dell’Angelica EC, Hartnell L, Bonifacino JS, Gahl WA. A new variant of Hermansky-Pudlak syndrome due to mutations in a...
gene responsible for vesicle formation. *Am J Med.* (2000) 108:423–27. doi: 10.1016/S0002-9343(99)00436-2

129. Yang W, Li C, Ward DM, Kaplan J, Mansour SL. Defective organellar membrane protein trafficking in Ap3b1-deficient cells. *J Cell Sci.* (2000) 113:4077–86.

130. Lawton AP, Prigozy TI, Brossay L, Pei B, Khurana A, Martin D, et al. The mouse CD1d cytoplasmic tail mediates CD1d trafficking and antigen presentation by adaptor protein 3-dependent and -independent mechanisms. *J Immunol.* (2005) 174:3179–86. doi: 10.4049/jimmunol.174.6.3179

131. Keller CW, Loi M, Ewert S, Quast I, Theiler R, Gannage M, et al. Identification of a homozygous deletion in the AP3B1 gene from AP-3-deficient mocha mice. *Cell Immunol.* (2001) 210:143–53. doi: 10.1006/cimm.2001.1817

132. Caplan S, Dell’Angelica EC, Gahl WA, Bonifacino JS. Trafficking of major histocompatibility complex class II molecules in human B-lymphoblasts deficient in the AP-3 adaptor complex. *Immunol Lett.* (2000) 72:113–7. doi: 10.1016/S0165-2478(00)00176-0

133. Pei B, Zhao M, Miller BC, Vela JL, Bruinsma MW, Virgin HW, et al. Invariant NKT cells require autophagy to coordinate proliferation and survival signals during differentiation. *J Immunol.* (2015) 194:5872–84. doi: 10.4049/jimmunol.1402154

134. Ververs FA, Kalkhoven E, Van’t Land B, Boes M, Schipper HS. Immunometabolic activation of invariant natural killer T cells. *Front Immunol.* (2018) 9:1192. doi: 10.3389/fimmu.2018.01192

135. Sugita M, Cao X, Watts GE, Rogers RA, Bonifacino JS, Brenner MB. Failure of trafficking and antigen presentation by CD1 in AP-3-deficient cells. *Immunity.* (2002) 16:697–706. doi: 10.1016/S1074-7613(02)00311-4

136. Sevilla LM, Richter SS, Miller J. Intracellular transport of MHC class II and associated invariant chain in antigen presenting cells from AP-3-deficient mocha mice. *Cell Immunol.* (2001) 210:143–53. doi: 10.1006/cimm.2001.1817

137. Caplan S, Dell’Angelica EC, Gahl WA, Bonifacino JS. Trafficking of major histocompatibility complex class II molecules in human B-lymphoblasts deficient in the AP-3 adaptor complex. *Immunol Lett.* (2000) 72:113–7. doi: 10.1016/S0165-2478(00)00176-0

138. Jung J, Bohn G, Allroth A, Boztug K, Khurana A, Martin D, et al. The mouse CD1d cytoplasmic tail mediates CD1d trafficking and antigen presentation by adaptor protein 3-dependent and -independent mechanisms. *J Immunol.* (2005) 174:3179–86. doi: 10.4049/jimmunol.174.6.3179

139. Keller CW, Loi M, Ewert S, Quast I, Theiler R, Gannage M, et al. Identification of a homozygous deletion in the AP3B1 gene from AP-3-deficient mocha mice. *Cell Immunol.* (2001) 210:143–53. doi: 10.1006/cimm.2001.1817

140. Yablonski D, Weiss A. Mechanisms of signaling by the hematopoieticspecific adaptor proteins, SLP-76 and LAT and their B cell counterpart, BLNK/SLP-65. *Adv Immunol.* (2001) 79:93–128. doi: 10.1016/S0091-6735(01)79003-7

141. Schwartzberg PL, Mueller KL, Qi H, Cannons JL. SLAM receptors and SAP influence lymphocyte interactions, development and function. *Nat Rev Immunol.* (2009) 9:39–46. doi: 10.1038/nri2456

142. Plas DR, Johnson R, Pingel JT, Matthews RJ, Dalton M, Roy G, et al. Direct regulation of ZAP-70 by SHP-1 in T cell antigen receptor signaling. *Science.* (1996) 272:1173–6. doi: 10.1126/science.272.5265.1173

143. Fawcett VC, Lorenz U. Localization of Src homology 2 domain-containing phosphatase 1 (SHP-1) to lipid rafts in T lymphocytes: functional implications and a role for the SHP-1 carboxyl terminus. *J Immunol.* (2005) 174:2849–59. doi: 10.4049/jimmunol.174.5.2849

144. Langlet C, Bernard AM, Drevot P, He HT. Membrane rafts and signaling by the multichain immune recognition receptors. *Curr Opin Immunol.* (2000) 12:250–5. doi: 10.1016/S0952-7915(00)00084-4

145. Boerth NJ, Sadler JJ, Bauer DE, Clements JL, Ghert SM, Koretzky GA. Recruitment of SLP-76 to the membrane and glycolipid-enriched membrane microdomains replaces the requirement for linker for activation of T cells in T cell receptor signaling. *J Exp Med.* (2000) 192:1047–58. doi: 10.1084/jem.192.7.1047

146. Kumar A, Pyarou K, Yarosz EL, Hong H, Lyssiotis CA, Giri S, et al. Enhanced oxidative phosphorylation in NKT cells is essential for their survival and function. *Proc Natl Acad Sci USA.* (2019) 116:7439–48. doi: 10.1073/pnas.1901376116

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Copyright © 2019 Gerth and Mattner. *This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.*