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Dickkopf1: An Immunomodulator in Tissue Injury, Inflammation, and Repair

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ABSTRACT

Upon injury, inflammation and repair processes are orchestrated to maintain tissue homeostasis. The Wnt ligands play essential roles in cell differentiation and proliferation for tissue repair and regeneration. It is increasingly clear that Wnt ligands play crucial immunemodulatory roles in inflammatory diseases. It is predicted that comprehensive research regarding the cross-talk between nonimmune and immune cells in tissue injury and repair will flourish. The Wnt system and immune system interaction will be critical to understanding tissue injury, inflammation, and repair. In this study, we will first introduce the Wnt system and review the role of the Wnt system in tissue regeneration and repair. We will review the previous literature regarding how the Wnt ligands regulate the immune system. Next, we will discuss the current and future perspectives of Wnt ligands to target cancer and other immunological diseases. Finally, we will discuss the quintessential Wnt antagonist Dickkopf1 as an immunomodulatory ligand. *ImmunoHorizons*, 2021, 5: 898–908.

INTRODUCTION

Wnt signaling plays an important role in developing and regenerating organs in the body. In mammalian cells, there are 19 Wnt ligands, 10 Frizzled (FZD) receptors, and several coreceptors (e.g., low-density lipoprotein receptor–related proteins [LRP] 5 and 6) (1). Wnt ligands are ∼40 kDa in size and have multiple cysteine residues. They are secreted as lipid-modified glycoproteins (2). The Wnt signaling pathway is broadly classified into the canonical and noncanonical Wnt pathways (3).

The canonical and noncanonical Wnt pathways have been extensively reviewed multiple times previously (4–7). Briefly, the canonical Wnt ligands (e.g., Wnt1, Wnt2, Wnt3, Wnt3a, and Wnt8a) bind to the FZD and its coreceptors LRP5 and LRP6. Transcriptional activation complex is formed with β-catenin, T cell–specific factor (TCF), and lymphoid enhancer–binding factor (LEF) (8, 9). The transcriptional activation complex induces the canonical Wnt target gene expression, such as *Axin2* and *Lgr5*, which participate in tissue regeneration by regulating cell proliferation and cell differentiation (3, 10).

The noncanonical Wnt ligands (e.g., Wnt4, Wnt5a, Wnt5b, Wnt6, Wnt7a, and Wnt11) activate the Wnt/planar cell polarity (PCP) or Wnt/Ca^{2+} pathways (1). In the Wnt/PCP pathway, Rho-associated kinase (ROCK) and JNK induces gene expressions for polarized cell migration and cytoskeletal rearrangement (1). In the Wnt/Ca^{2+} pathway, the noncanonical Wnt ligands induce gene expressions for cell fate and cell migration (11). The summary of the canonical and noncanonical Wnt pathways is shown in Fig. 1.

Recently, the signaling cross-talk between the Wnt/β-catenin pathway and other pathways (e.g., Jak–STAT cytokine pathway,

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Abbreviations used in this article: AT2, alveolar type 2; DC, dendritic cell; DKK, Dickkopf; *Dkk1*$^{d/d}$, DKK1 hypomorphic *doubleridge*; FZD, Frizzled; HDM, house dust mite; LEF, lymphoid enhancer–binding factor; LRP, low-density lipoprotein receptor–related protein; MDSC, myeloid-derived suppressor cell; PCP, planar cell polarity; ROCK, Rho-associated kinase; TCF, T cell–specific factor; Treg, regulatory T cell; tTreg, thymically derived regulatory T cell.

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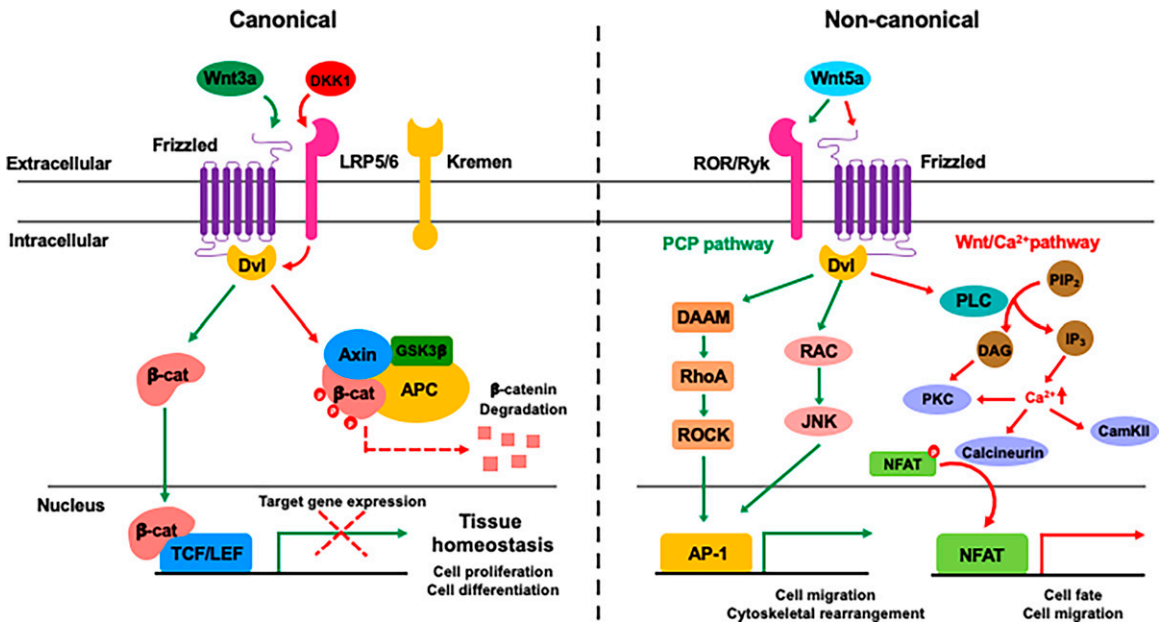

FIGURE 1. Wnt signaling in tissue injury and repair.

Upon the canonical Wnt ligands (e.g., Wnt3a) binding to the FZD and its coreceptor LRP5/6, Dvl and β-catenin destruction complex to the LRP6 is initiated. β-Catenin accumulates in the cytoplasm and then enters the nucleus to form a transcriptional activation complex with TCF and LEF. The transcriptional activation complex induces the canonical Wnt target gene expression for cell proliferation and cell differentiation. DKK1 is a competitive inhibitor against Wnt3a for LRP5/6, potentially inhibiting the Wnt-mediated tissue homeostasis. In the absence of Wnt3a, β-catenin is phosphorylated and degraded by the β-catenin destruction complex, diminishing canonical Wnt target gene expression. The noncanonical Wnt ligands (e.g., Wnt5a) activate the Wnt/PCP or Wnt/Ca^{2+} pathways. In the PCP pathway, Wnt ligands stimulate the activation of the small GTPase Rho and Rac, inducing ROCK and JNK, respectively. ROCK and JNK induce gene expressions for polarized cell migration and cytoskeletal rearrangement. In the Wnt/Ca^{2+} pathway, activated PLC stimulates 1,2-diacylglycerol (DAG) and inositol 1,4,5-triphosphate (IP_3). IP_3 triggers Ca^{2+} release in the cytoplasm and activates effector molecules, such as protein kinase C (PKC), CamKII, and calcineurin. Calcineurin activates a transcriptional factor, NFAT, via dephosphorylation.

the TGF-β pathway, the DNA damage pathway, and the Hippo pathway) has been suggested (12–16). These studies warrant the identification and elucidation of context-dependent signaling pathways in various cellular contexts, including immune cells.

Several conserved antagonists regulate Wnt signaling. Five classes of Wnt antagonistic ligands were identified. The secreted FZD-related proteins (SFRP) 1–5, Wnt inhibitory factor (WIF), Cerberus, Wise/Sclerostin (Wise/SOST), and the Dickkopf (DKK) family proteins (DKK1–4) inhibit Wnt pathways (17). SFRPs and WIF1 bind to Wnt agonistic ligands in the extracellular space, sequestering Wnt agonists to inhibit both canonical and noncanonical Wnt signaling. DKK family proteins and Wise/SOST competitively inhibit the binding of Wnt agonists to their receptor LRP5/6 (18). Among DKK family proteins, DKK1 is a quintessential Wnt antagonist and the most extensively studied DKK protein out of four members (DKK1, -2, -3, and -4) (19).

DKK1 is a competitive inhibitor against Wnt3a, inhibiting the Wnt-mediated tissue repair process (20). DKK1 was first described as an essential molecule to promote Spemann's head formation activity in *Xenopus laevis* embryos (21). DKK1 knockout mice showed incomplete development of anterior midbrain structures, resulting in neonatal death (22). DKK1 hypomorphic *doubleridge* (*Dkk1*$^{d/d}$) mice were previously generated, and these mice showed a systemic reduction in DKK1 expression with blinded eyes (23). The binding of DKK1 to its receptor LRP6 and Kremen1 has been well characterized by multiple studies, suggesting a dose-dependent inhibition of the canonical Wnt signaling pathway by DKK1 requires multiple protein interactions (24–27). Krm proteins are necessary to increase the availability of the LRP5/6 complex in the cell surface, elevating the inhibitory potential of DKK1 against Wnt3a (28). In the immune system, the role of DKK1 in thymocytes development was demonstrated by overexpression of murine DKK1 in fetal thymic organ culture (29). In this study, a variety of Wnt ligands and receptor expressions were surveyed at different thymocyte developmental stages. DKK1 overexpression halted thymocytes development at the very early step of the double negative 1 stage, suggesting an important role of DKK1 in thymocytes development. Although DKK1 has been primarily identified as a Wnt antagonist, alternative signaling pathways induced by DKK1 were identified recently. For example, a recent study showed that cytoskeletal-associated protein (CKAP)–4 is a receptor for DKK1 (30). This study showed that DKK1 induces cell proliferation via the AKT/PI3K pathway in

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normal and cancer cells independent of the canonical Wnt signaling pathway activation. In line with the signaling cross-talk between the Wnt pathway and other signaling pathways, the context-dependent roles of DKK1 and their mechanistic details require more detailed analyses in the future.

WNT SIGNALING IN TISSUE REPAIR AFTER INJURY

Recent studies reported that Wnt signaling is initiated in response to organ or tissue injuries (e.g., skin wound, bone fracture, retinal and intestine injury, chronic liver injury, lung injury, and acute kidney injury) (31). The Wnt signaling induces the proliferation of stem cell and local progenitor cell populations. Subsequently, stem cells are differentiated into multiple cell types to contribute to tissue regeneration and remodeling (10).

The role of Wnt signaling has been emphasized for its critical role in stem cell differentiation upon tissue injury. During skin puncture wound repair in rats, the Wnt/β-catenin pathway stimulates the proliferation of epidermal stem cells (32). The expanded epidermal stem cells migrate to the wound site and differentiate into keratinocytes that reconstruct the epidermal barrier (32). It has been shown that the β-catenin protein level is upregulated during bone fracture healing in humans and mice (33). In this study, it has been demonstrated that β-catenin is required for mesenchymal stem cells to differentiate to either osteoblasts or chondrocytes in the mouse bone fracture model using β-catenin–deficient mice.

A previous study has shown that Wnt/β-catenin signaling promotes the proliferation of Müller glia–derived retinal progenitors after retinal injury (34). *N*-methyl-D-aspartate receptor was injected to induce retinal damage in the mouse retina. Wnt3a promoted the proliferation of retinal progenitors in an *N*-methyl-D-aspartate–induced retinal injury mouse model and a genetic mouse model of retinal degeneration (*rd* mice).

Another study showed that the reduced expression of the Wnt antagonist DKK1 in mice increased the proliferation of colonic epithelial cells (35). DKK1 hypomorphic *Dkk1*$^{d/d}$ mice recovered markedly faster with reduced apoptosis of intestinal epithelial cells in murine dextran sodium sulfate colitis model.

Wntless (Wls) protein activates hepatic progenitor cells in murine models of chronic liver disease (36). Chronic liver injury was induced by giving L-thioacetamide in drinking water and a choline-deficient, ethionine-supplemented diet. Wls depletion in macrophages exacerbates L-thioacetamide– and choline-deficient, ethionine-supplemented–induced fibrosis. Another liver injury model using hepatic ischemia/reperfusion in rats showed that upregulation of the Wnt/β-catenin pathway by a Wnt agonist (BML-284) promoted hepatocyte proliferation to repair damaged liver tissues (37).

Upon acute kidney injury, the Wnt/β-catenin pathway was activated to integrate exogenous embryonic renal progenitor cells into damaged renal tubules, leading to kidney repair (38). When embryonic renal progenitor cells were treated with a β-catenin/TCF pathway inhibitor (IWR-1) prior to infusion into glycerol-injured mice, tubular integration of cells was reduced. After integration into damaged renal tubules, renal progenitor cells expressed Wnt4 during renal regeneration.

In the lung, it has been shown that Wnt-responsive, Axin2$^+$ alveolar epithelial progenitors within the alveolar type 2 (AT2) cell population expand rapidly to regenerate a large proportion of the alveolar epithelium after acute lung injury by PR8 H1N1 influenza virus infection in mice (39). Other studies also demonstrated that the Wnt-responsive AT2 cell population is activated by bleomycin-induced lung injury in mice.

Within the alveoli of the normal adult lung, the canonical Wnt signaling pathway in the AT2 cell population is activated after bleomycin-induced injury (40, 41). Collectively, these studies suggest that multiple organs use Wnt signaling to restore tissue homeostasis upon various types of injuries that accompany immune responses. This raises two important questions. First, how do Wnt ligands augment the signaling pathways? Second, because the Wnt signaling pathway is crucial for tissue repair and regeneration, would Wnt antagonists cause pathological inflammation? If so, what are the mechanisms of such pathological disease outcomes?

WNT SIGNALING REGULATES IMMUNE CELLS

Although Wnt signaling pathways and their roles in nonimmune cells, including stem cells, have been studied actively, recent findings suggest that the role of Wnt ligands is significant in regulating immune cells and their immunomodulatory mechanisms (42).

The canonical Wnt signaling proteins exert their roles in T cell differentiation and effector function in various inflammatory diseases, including cancer, autoimmunity, and viral infections (42). The CD4$^+$ and CD8$^+$ T cell–mediated immune responses are crucial for adaptive immunity upon tissue injury. The differentiation and persistence of memory CD8$^+$ T cells are regulated by Wnt pathway transcriptional factors, such as TCF-1 and LEF-1 in acute viral infection models in mice (43, 44). The indispensable role of TCF-1 has been studied in T cell development and CD8$^+$ T cells. TCF-1 is expressed at the CD4CD8 double-negative thymocytes upon Notch signaling, and in turn, TCF-1 induces Gata-3 to direct T cell–lineage commitment with β-catenin. CD8 single-positive T cells are generated by the interaction of TCF-1/LEF-1 with Runx3 interaction repressing *Cd4*, *Foxp3*, and *Rorc* gene expression by intrinsic histone deacetylase activity TCF-1 and LEF-1 (45–48). Among multiple isoforms of TCF-1, it has been shown that the long isoform TCF-1–deficient mice (p45$^{-/-}$) are vital for thymocytes survival via the interaction between β-catenin and TCF-1 via its N-terminal β-catenin interaction domain (49). TCF-1 long isoform (p45$^{-/-}$)–deficient mice revealed that TCF-1 short isoforms that lacked β-catenin domain were sufficient to support thymocyte development and regulate most TCF-1–mediated gene regulation (49, 50). The use of TCF-1 long isoform-deficient mice in the acute lymphocytic choriomeningitis virus infection model demonstrated its importance in central

memory $CD8^+$ T cell maturation and secondary expansion (51). The TCF-1's role in memory $CD8^+$ T cell generation and effector function was tested in the *Listeria monocytogenes* infection and lymphocytic choriomeningitis virus infection model. The effector-prone daughter cells with more robust proliferation become TCF-1^{neg} cells. The daughter cells showed less proliferation with harbor self-renewal activity to become TCF-1^{pos} cells postinfection resolution (52). The gain-of-function and the loss-of-function analyses of TCF-1 in thymically derived regulatory T cells (tTreg) showed that TCF-1 hampers the ability of regulatory T cell (Treg) precursors to differentiate into tTregs, and Foxp3 represses TCF-1 function in tTregs (53). In the periphery, Foxp3-mediated Treg function was abolished by TCF-1 (54). The study showed that Wnt3a activates the canonical Wnt pathway signaling in Tregs via TCF-1, inhibiting the Foxp3-mediated gene expression program. A global chromatin immunoprecipitation indicated that TCF-1 and Foxp3 share their target genes considerably. The immunomodulatory roles of Wnt ligands have been studied in $CD4^+$ T cells. In naive $CD4^+$ T cells from human cord blood, Wnt3a promoted Th2 cell differentiation (55). Wnt5a induces chemokine CXCL12 and is required for T cell migration (56).

Wnt ligands and Wnt signaling components regulate dendritic cells (DCs). DCs modulate innate immune and adaptive responses in various types of inflammatory diseases. Wnt ligands such as Wnt3a and Wnt5a induce tolerogenic DCs to suppress inflammation in response to LPS (57, 58). Activation of β-catenin regulates intestinal DCs to a tolerogenic state, limiting the inflammatory response in the murine dextran sodium sulfate colitis model (59). DC-derived Wnt ligands can modulate B cell– and T cell–mediated immune responses. For example, follicular DCs from human tonsils can express Wnt5a. Follicular DC–derived Wnt5a protects germinal center B cell death (60).

The relationship of the Wnt signaling in macrophages has emerged in tissue injury, repair, and regeneration. Macrophages exhibit crucial homeostatic activity in the most immunologically active lung, intestine, liver, kidney, and heart. Typically, by simplified classification, macrophages are primarily divided into two major groups, classically activated macrophages (M1-like macrophages) and alternatively activated macrophages (M2-like macrophages), based on functions and expression patterns of genes and proteins. M1-like macrophages release proinflammatory cytokines to promote inflammation. M2-like macrophages secrete anti-inflammatory cytokines, which reduce inflammation and contribute to cell growth and tissue repair.

Wnt signaling regulates macrophages for lung regeneration upon the injury. For example, Wnt6 induced M2-like macrophage polarization and promoted macrophage proliferation in the lung in *Mycobacterium tuberculosis*–infected mice (61). In cigarette smoke extract–challenged mouse models, increased Wnt5a protein levels in the lung influenced the M1/M2 macrophage polarization (62). Wnt/β-catenin signaling was activated in chronic obstructive pulmonary disease patient lungs, resulting in altered macrophage activity and elastin-mediated lung tissue remodeling (63). A chronic obstructive pulmonary disease study using the insulin resistance rat model has shown that Wnt5a/JNK1 signaling pathway was activated and induced lung macrophage activation (64).

Wnt signaling in macrophages also plays an essential role in intestinal epithelial proliferation and differentiation. Recently, the role of macrophage-derived Wnts in intestinal repair has been studied. Following radiation injury in the intestine, Wnt ligands were expressed by bone marrow–derived macrophages and secreted through extracellular vesicles, rescuing intestinal stem cells (65). M2-like macrophages significantly increased and acted as a source of Wnt1, which reduced alkaline phosphatase activity in the mucosa of ulcerative colitis patients (66). Alkaline phosphatase activity is an established marker of intestinal epithelial cell differentiation, and thus, this indicates that ulcerative colitis and Wnt ligand expression from macrophages is negatively associated. It should be noted that this direct link has been demonstrated only in cell lines in vitro. Further studies are warranted to demonstrate more physiologic importance.

In response to injury, Wnt ligands derived from macrophages are required for tissue regeneration in the liver, kidney, and heart. In the liver, macrophage-derived Wnt ligands activated proregenerative hepatic progenitor cells and showed antifibrotic potential in murine models of chronic liver disease (36). Wnt3a produced by macrophages induced hepatic progenitor cell proliferation in the progression of pediatric nonalcoholic fatty liver disease (67). Macrophage-derived Wnt ligand is also critical for kidney repair and regeneration. Wnt7b produced by macrophages promoted kidney regeneration by directing epithelial cell-cycle progression (68). After myocardial infarction, macrophages selectively upregulate the noncanonical Wnt pathway to improve cardiac healing and function (69). Overall, Wnt ligands in macrophages play reparative roles in tissue injury and repair of most organs.

The relationship between proliferative and inflammatory phenotypes of tissue macrophages was recently characterized by investigating the Wnt/β-catenin pathway in alveolar macrophages in mice (70). In this study, Wnt/β-catenin pathway activation promoted the inflammatory activity of alveolar macrophages while inhibiting their proliferation and stemness in a murine influenza viral pneumonia model. Wnt treatment promoted β-catenin–HIF-1α interaction and glycolysis-dependent inflammation, contributing to higher morbidity upon viral infection. Taken together, these studies indicate that the role of Wnt ligands is context dependent with each tissue and injury model used. This is a rapidly evolving field given that there are multiple Wnt ligands and the signaling mechanisms are far from fully understood.

DKK1 AS AN IMMUNE MODULATOR IN PATHOLOGICAL IMMUNE RESPONSES

DKK1 as a protumorigenic ligand

The involvement of DKK1 in human diseases was implicated in cancer and bone pathology (20). DKK1's role as an immunomodulator was recently identified by multiple groups, suggesting that DKK1 can be a promising molecular link between the Wnt and

immune systems. One of the most actively studied areas is the role of DKK1 as an immunosuppressor in cancer.

A recent study showed that inhibition of canonical Wnt signaling through DKK1 secreted from tumor cells imposed a slow-cycling state on latency-competent cancer cells along with extensive downregulation of UL16-binding proteins (ULBPs), which are human ligands for NK cell–activating receptor NKG2D. This resulted in immune evasion against NK cell–mediated immune surveillance, promoted long-term survival, and preserved cancer-initiating capacity in the latency-competent cancer cells using DKK1 during the latent metastasis stage (71). Another study showed that systemic levels of DKK1 were increased in the bone microenvironment in Lewis lung carcinoma, B16 melanoma, and murine pancreatic ductal adenocarcinoma models. It has been demonstrated that DKK1 targets the β-catenin–mediated Wnt signaling pathway in myeloid-derived suppressor cells (MDSCs) in both mice and humans, resulting in immune suppression in the tumor microenvironment. DKK1 Ab treatment showed a significant decrease in MDSC accumulation and tumor growth by rescuing β-catenin expression in MDSCs and restoring T cell recruitment at the tumor site (72). In addition, the role of DKK1 in organ-specific metastasis was identified recently (73). The study showed that the expression of DKK1 in the breast cancer cell line SCP28 suppressed lung metastasis and macrophage/neutrophil recruitment via inhibiting the noncanonical Wnt/PCP–RAC1–JNK signaling pathway in the cancer cells. DKK1 accelerated bone metastasis via controlling canonical Wnt signaling of osteoblasts. The immunosuppressive role of DKK1 in these studies is summarized in Table I.

A very recent study demonstrated the efficacy of murinized anti-DKK1 Ab (mDKN-01) in murine melanoma and 4T1 metastatic breast cancer models. The study showed that NK cells activated by IL-15 and IL-33 were required for tumor growth inhibition and reduced the number of MDSCs and their PDL-1 expression levels in the tumor (74). These studies consistently point out that DKK1 is a protumorigenic ligand to facilitate immune evasion by negatively regulating multiple types of immune cells.

DKK1 in pathological inflammatory diseases

Although the protumorigenic and immune-evasive role of DKK1 has been studied, there are relatively limited amounts of studies that addressed the immunomodulatory functions of DKK1.

Earlier studies showed that infectious pathogens induce DKK1. For instance, a previous study showed that *Aspergillus fumigatus* markedly induced the platelet activation marker P-selectin (CD62P) and the release of DKK1 from platelets in vitro. The study showed that the hypha-mediated activation of platelets increased the release of proinflammatory cytokine IL-8 in THP-1 monocytes and human adherent monocytes (75). Another study showed that DKK1 from HUVECs caused the proinflammation to secrete IL-6 and IL-8 in response to a Gram-negative bacterial pathogen, *Rickettsia conorii* (76). DKK1 small interfering RNA transduction in HUVECs significantly attenuated the inflammatory responses to *R. conorii* with reduced expression levels of IL-6, IL-8, and growth-related oncogene (GRO)–α at both mRNA and protein levels.

Recently, it has been shown that elevated systemic levels of DKK1 protein were observed in the murine house dust mite (HDM)–induced asthma model and chronic cutaneous *Leishmania* skin infection model (77). In this study, DKK1 promoted pathological type 2 inflammation via CD4 T cells, arguing that platelets are a primary source of DKK1. Interestingly, they demonstrated that *Dkk1*$^{d/d}$ mice and DKK1 inhibitor (WAY-262611) significantly reduced the interaction between platelets and leukocytes in peripheral blood in the two type 2 cell–mediated pathological inflammation model.

Another study demonstrated that platelet-derived DKK1 inhibited the Wnt/β-catenin signaling pathway in alveolar epithelial cells in an influenza virus infection model (78). In this study, the activation of Wnt/β-catenin signaling pathway downregulated adhesion molecules such as ICAM-1/VCAM-1, reducing the interaction between alveolar epithelial cells and neutrophils/macrophages. Other studies showed that DKK1 promotes pathological inflammation in a highly prevalent infection-triggered inflammatory disease that results in bone loss (79).

The deletion of DKK1 in osteocytes using Dmp1Cre-DKK1$^{fl/fl}$ mice prevented periodontitis-induced alveolar bone loss. Dmp1-Cre-DKK1$^{fl/fl}$ mice were protected from increased inflammatory infiltrates and TNF and IL-1 expressions in the gingiva and the increased numbers of osteoclasts. Collectively, these studies suggest that DKK1 has various roles in inflammatory diseases and requires further studies to elucidate the immunomodulatory

TABLE I. DKK1's role as an immunosuppressor in cancer

Cancer Cell	Mechanism of Action	Immunosuppression	Effect	Ref.
Human lung cancer cells (H2087) Human breast cancer cells (HCC1954)	Downregulation of ligands (ULBPs) for NK cell–activating receptor NKG2D	Immune evasion against NK cell–mediated immune surveillance	Long-term survival and preserved cancer-initiating capacity	71
Mouse Lewis lung carcinoma cells	Downregulation of β-catenin expression in MDSCs	Decreased T cell recruitment	Decrease in MDSC accumulation and tumor growth by neutralizing DKK1	72
Breast cancer cells (SCP28)	Inhibition of the noncanonical Wnt/PCP-RAC1-JNK and Wnt/Ca^{2+}-CaMKII-NF-κB signaling pathway	Suppressed macrophage/neutrophil recruitment	Tumor proliferation and promoted breast-to-bone metastasis	73

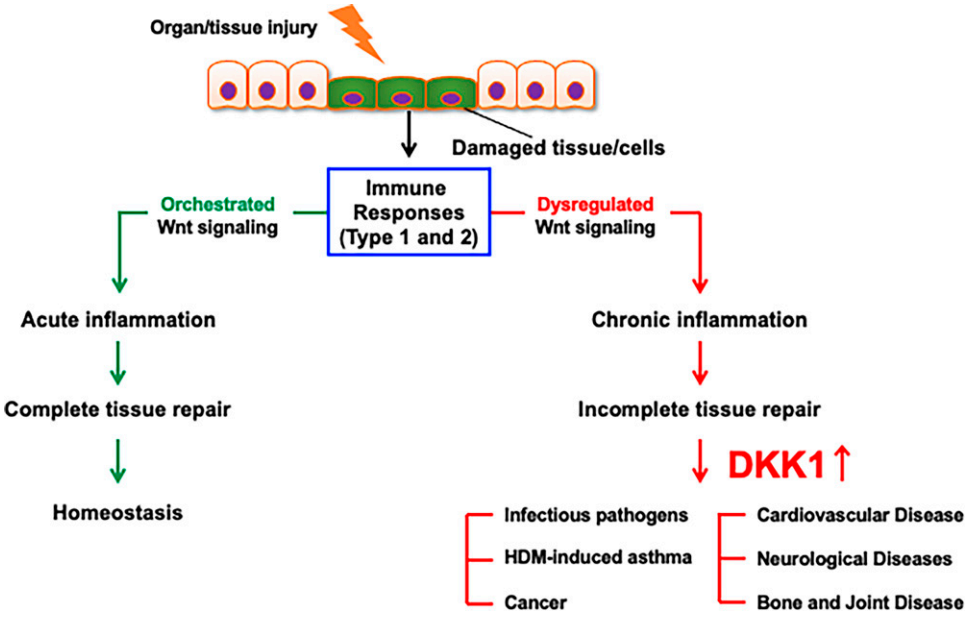

FIGURE 2. DKK1-mediated inflammatory diseases.

Multiple types of injuries augment DKK1-mediated inflammation. Upon injury, inflammation is initiated while the tissue repair process is coordinated with the Wnt system. Once the two systems are organized promptly, tissue repair processes are completed. The elevation of DKK1 expression levels was identified. The mechanisms of DKK1 expression in the listed chronic inflammatory diseases have been addressed in infectious pathogens [e.g., *L. major* (77), *Candida albicans* (95), influenza virus (78), and *R. conorii* (76), HDM-induced asthma (77), cancer (71, 73), cardiovascular diseases (e.g., atherosclerosis) (96), neurologic diseases (e.g., Alzheimer disease) (97), and bone and joint disease (79)]. Mechanistic studies on the causal role of DKK1 in other chronic inflammatory diseases are warranted.

functions of DKK1. The different types of DKK1-mediated pathological disorders are shown in Fig. 2.

DKK1 in immunological diseases on the horizon

The studies mentioned above make it increasingly clear that DKK1 can be an important immunomodulator in multiple inflammatory diseases. With its role as a Wnt antagonist, DKK1 may inhibit tissue repair while acting as an important immunomodulator. Neutralization of DKK1 is being investigated in clinical trials and mouse models of cancer to use it as an immune checkpoint target strategy. A recent study tested whether DKK1-neutralizing IgG4 Ab DKN-01 can show antitumor activity with esophagogastric cancer patients with high expression of DKK1 (80). The study demonstrated that DKN-01 monotherapy was well tolerated, and DKN-01 therapy was effective when combined with pembrolizumab in a small cohort. DKK1 was identified as a biomarker for esophagogastric cancer. Further studies are warranted with larger cohorts. However, we are far from a comprehensive understanding regarding how DKK1 plays a crucial immunomodulator and, more importantly, how the Wnt system interacts with the immune system.

Understanding how DKK1 and other Wnt ligands function as crucial immunomodulators hinge on holistic approaches and a thorough investigation with multiple models and cell types. Although cancer immunology is undoubtedly an important and exciting area, other important immunological diseases will be of interest. A few lessons from previous studies regarding DKK1 could be considered for future studies for other Wnt ligands.

First, the role of DKK1 depends on cellular and molecular context. Often DKK1 is considered as a Wnt antagonist. However, novel signaling pathways are augmented by DKK1 even without Wnt agonists. Each inflammatory disease needs to be examined with careful assessment on the microenvironment and cell types of interest in humans and mice. For instance, the proinflammatory and protumorigenic roles of DKK1 have been identified in multiple diseases in humans and mice. However, our previous study suggested that Treg cell–derived DKK1 can prevent T cell–mediated autoimmune colitis model in mice (81).

Interestingly, this study showed that DKK1 is anti-inflammatory, and DKK1 was present in Treg as a membrane-bound form. The study has a limitation in that the host is immunodeficient mice to allow homeostatic expansion of Tregs, and DKK1 expression is upregulated upon strong TCR stimulation. Nevertheless, this shows that the role of DKK1 needs to be determined after thorough consideration of molecular and cellular context.

Another example could be a recent study regarding radiation-induced injury that showed that the systemic administration of DKK1 to irradiated mice promoted survival rate along with hematopoietic recovery in mice (82). The inducible deletion of one allele of the *Dkk1* gene in Osterix (Osx)–expressing cells in mice inhibited bone marrow stem and progenitor cell

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repopulation and complete blood count levels after irradiation. In this study, DKK1 decreased mitochondrial ROS levels, suppressed senescence, and indirectly induced epidermal growth factor (EGF) secretion, claiming DKK1 as a regulator for HSC regeneration. The follow-up study implicated a therapeutic potential of DKK1-treated endothelial progenitor cells to rescue acute radiation injury–mediated damages in a murine allograft transplantation model (83). These studies suggest that DKK1 can promote tissue repair and anti-inflammatory, implying that the cellular and molecular context determines its role. Further molecular characterization of DKK1 and DKK1-mediated signaling pathways should be investigated to understand these discrepancies more in detail.

Second, DKK1 may be secreted from multiple sources. Although platelets are a primary source of circulating DKK1 in homeostatic conditions and inflammatory lung diseases and infectious pathogens, tumor cells secrete DKK1 in the tumor microenvironment. Previous studies also showed that DKK1-expressing cells could vary. Thus, the cellular and molecular mechanisms of DKK1 expression clearly need further investigation to elucidate further DKK1-mediated pathological inflammation and tissue regeneration/repair in a given context. For instance, the study mentioned that Dmp1Cre-DKK1$^{fl/fl}$ mice showed a protective effect from alveolar bone loss, whereas T cell–specific deletion of DKK1 protected mice from ovariectomy-induced bone loss (84). These results lead to a question regarding the molecular mechanism of DKK1 expression in a given cell type and raise a question about how each cell type can sense extracellular cues to induce DKK1 expression.

Third, the intensity, specificity, and duration of DKK1-mediated immune responses may determine the chronicity and severity of inflammation. A previous study suggested that type 2 cell–mediated inflammation was boosted by DKK1 in the acute allergen challenge model using HDM and chronic skin infection model using *Leishmania major* (77). Notably, not all pathogen-associated molecular patterns could induce the elevation of DKK1, suggesting that DKK1 may be generated by specific tissue/organ challenges. It has also been shown that DKK1 was induced in the acute influenza virus infection model in mice (78).

Other DKK proteins in inflammatory diseases

Other DKK proteins' immunomodulatory functions have been demonstrated. DKK3 has been studied in multiple inflammatory diseases models, whereas DKK2 and DKK4 were not extensively studied.

DKK3-deficient mice showed that plasma IgM levels were increased, and NK cell numbers were increased in the homeostatic condition compared with littermate control mice (85). Interestingly, the canonical signaling pathway has not been altered. Unlike DKK1, previous studies showed that DKK3 elicited antitumor immune responses. Recombinant DKK3 treatment on mouse bone marrow–derived DCs and splenocytes increased Th1 cell polarization by increasing CD40, CD80, and IFN-γ expression levels (86). Another study showed that DKK3 differentiates monocytes into DC-like cells via STAT3 and STAT5 phosphorylation (87). The i.p. injection of recombinant DKK3 into the renal cell adenocarcinoma-bearing mice inhibited tumor growth, suggesting antitumorigenic immune-modulatory roles of DKK3. These findings imply that DKK3 can potentiate antitumor immune responses through JAK–STAT pathways.

It should be noted that the role of DKK3 is dependent on molecular and cellular context. For example, a previous study showed tolerogenic or immunosuppressive functions of DKK3. In a double-transgenic mouse model in which a parenchymal self-antigen neonatally tolerizes CD8$^+$ T cells, DKK3 was required to maintain CD8$^+$ T cell tolerance to suppress Ag-specific T cell activation (88). The study further demonstrated that Ab-mediated neutralization DKK3 enhanced CD8$^+$ T cell proliferation and IL-2 production in vitro and led to tumor regression in vivo. Ab-mediated DKK3 neutralization and DKK3 knockout exacerbated experimental autoimmune encephalomyelitis with increased numbers of tissue-infiltrating T cells in mice (89). Immunosuppressive roles of DKK3 were also identified in murine models of fibrosis and pancreatic ductal adenocarcinoma (90, 91).

For DKK2, very little is known about its immunomodulatory roles. Recently, it has been demonstrated that administration of DKK2 neutralizing Ab in MC38 colon cancer model showed enhanced cytotoxicity of tumor-infiltrating CD8$^+$ T cells and NK cells (92). Similar to a study showing that DKK1 used STAT6 to promote type 2 inflammation, the study demonstrated that DKK2 binds to LRP5 but not LRP6 in CD8$^+$ T cells and NK cells and inhibits p-STAT5–mediated granzyme B expression. Other studies further supported the suppressive roles of DKK2 on antitumor immunity in the *Apc* knockout and *Kras*^{G12D} mutation-driven colorectal cancer and lung cancer models (93, 94). Collectively, these studies suggest that the immunomodulatory roles of other DKK proteins need to be studied in a context-dependent manner, and more detailed studies are warranted.

PERSPECTIVE: WNT-MEDIATED IMMUNE REGULATION ON THE HORIZON

The past decade has seen a remarkable increase in studies regarding the role of Wnt ligands in cancer and other chronic inflammatory diseases. Yet, it remains elusive to integrate these pieces of information, establishing a big overarching question in the immunology field. The number of Wnt ligands, posttranslational modifications of Wnt ligands, their receptors, and downstream signaling pathways in different immune and nonimmune cells require further studies to delineate and identify new immunological targets for therapeutic intervention. Most in vitro experimental settings in these studies had limitations to understanding the physiologic binding of DKK1 in vivo. Posttranslational modifications of Wnt ligands and antagonists, their binding capability, and the subsequent physiological outcomes in the inflammatory microenvironment are still largely unknown.

The recent technological advances in genomics, proteomics, and metabolomics approaches will enable us to identify potential molecular and cellular mechanisms, whereas traditional genetic, pharmacological, and immunological approaches will verify them. The advancement of other biomedical science fields will come together to decipher the Wnt-mediated regulation of tissue injury, inflammation, and repair comprehensively.

CONCLUSIONS

There has been a marked increase in studies regarding the role of Wnt ligands in immunology. DKK1 is a novel immunomodulatory protein in tissue inflammation and repair upon a variety of injuries. More detailed analyses on context-dependent roles of DKK1 will reveal new insights to understand inflammatory diseases and provide attractive therapeutic strategies.

DISCLOSURES

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