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cytokine BULLETIN

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INSIDE

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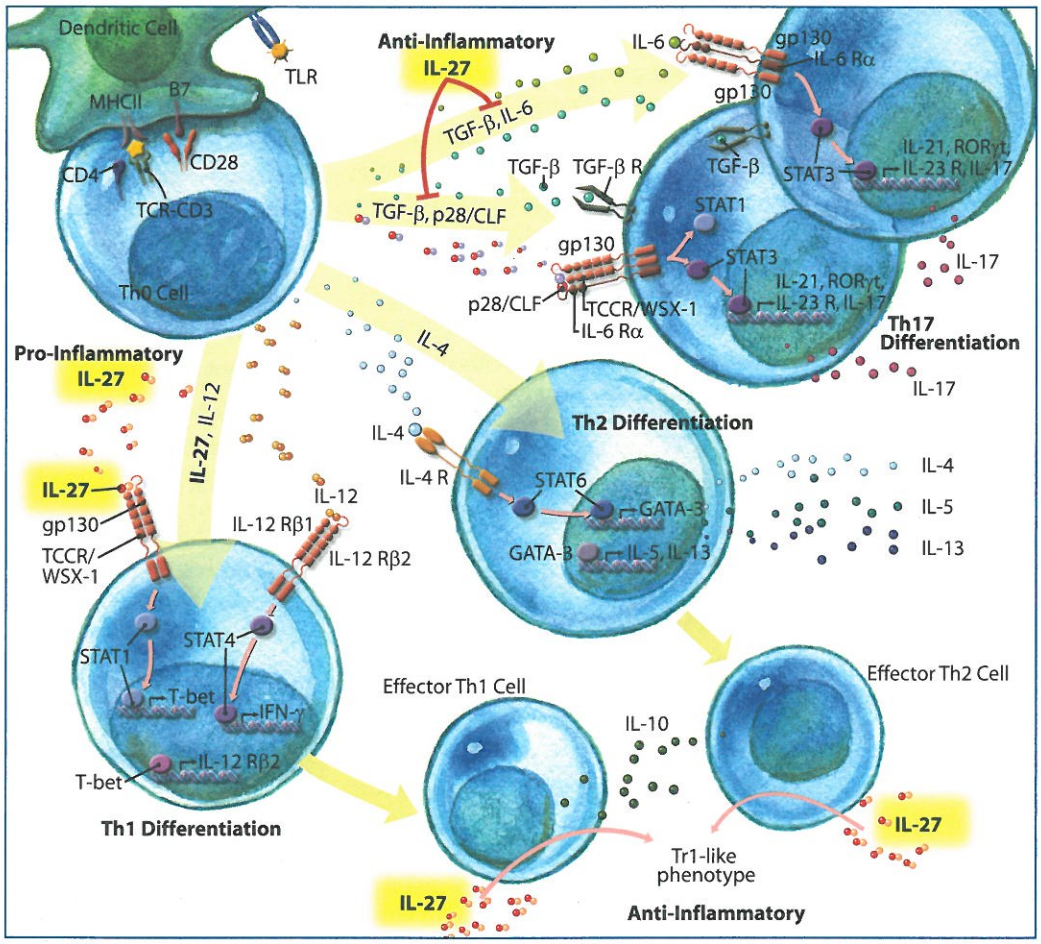

IL-27 Has Both Pro- and Anti-Inflammatory Effects. IL-27 is a heterodimeric cytokine secreted by activated antigen-presenting cells. It drives inflammation by promoting the early commitment of naïve CD4+ T cells to a Th1-specific lineage. In contrast, it inhibits inflammation by suppressing Th17 differentiation and inducing a T regulatory (Tr1)-like activity in differentiated Th1 and Th2 effector cells. IL-10 secretion by these cells has anti-inflammatory, immunosuppressive effects that may serve as a negative feedback mechanism to balance IL-27-induced Th1 differentiation. Recent evidence suggests that the p28 subunit of IL-27 may form a second protein complex with cytokine-like factor 1 (CLF), which is also involved in regulating the balance between pro- and anti-inflammatory T cell responses.

IL-27: Balancing T Cell-Mediated Immune Responses

Interleukin-27 (IL-27) is a member of the IL-12 family of heterodimeric cytokines that also includes IL-12, IL-23, and IL-35. Each of these cytokines consists of an alpha (p19, p28, or p35) and a beta (p40 or EBI3) chain, and signals through receptors that are highly expressed on T cells and/or natural killer cells. IL-27 is comprised of p28, a polypeptide related to IL-12 p35, and EBI3 (Epstein-Barr virus-induced gene 3), an IL-12/IL-23 p40-related protein.[1] It binds to a heterodimeric receptor complex formed by TCCR/WSX-1 and gp130, a common receptor subunit shared by IL-6 family cytokines.[2] Upon secretion by activated antigen-presenting cells, IL-27 promotes the expansion of naïve CD4+ T cells, and drives Th1 differentiation by inducing the expression of the Th1-specific transcription factor, T-bet.[2-4] At the same time, IL-27 in the absence of IL-4 inhibits the expression of the Th2-specific transcription factor, GATA-3, and suppresses Th2 cytokine production.[3,5] Despite its role in promoting Th1 differentiation, studies performed using TCCR/WSX-1-deficient mice infected with various pathogens suggest that IL-27 signaling is also required to prevent excessive T cell activity and limit pro-inflammatory cytokine production.[6-7] The importance of the anti-inflammatory properties of IL-27 was highlighted in 2006 when Batten *et al.* and Stumhofer *et al.* demonstrated that IL-27 could suppress the development of IL-17-producing Th17 cells.[8,9] Together these studies indicated that IL-27 may be important for inhibiting the pathogenesis of Th17-related inflammatory/autoimmune diseases.

Continued on page 7

Vitamin D Deficiency as a Risk Factor for Multiple Sclerosis

Multiple sclerosis (MS) is an autoimmune neurological disorder that affects over 2 million people worldwide. Onset of the disease is thought to occur following exposure of genetically predisposed individuals to an unknown environmental trigger that activates myelin-specific T cells. These cells cross the blood-brain barrier to trigger an inflammatory attack that demyelinates axons in the central nervous system.[1-3] Polymorphisms in the vitamin D receptor are among the genetic variations that increase the risk of MS. The incidence of MS is also elevated in northern climates, where vitamin D deficiency is common. Since exposure of the skin to ultraviolet light aids in the conversion of inactive vitamin D to the active form, $1,25(OH)_2$VitD3 (VitD3), the short days, low UV intensity, and lack of exposed skin common to winter months, decrease the amount of active VitD3 that can be produced.[1,2] Although the correlation between VitD3 deficiency and MS is intriguing, we are only now beginning to understand the biology that may explain this connection. In both human MS and in experimental autoimmune encephalomyelitis (EAE), the mouse model of the disease, VitD3 can influence specialized populations of cells that may play a critical role in protection against the disease. VitD3 promotes the formation of tolerogenic or "semi-mature" dendritic cells (DCs).[2] These DCs induce the development of $CD25^-FoxP3^{+/-}IL\text{-}10^{++}$ Tr1 cells, a subset of regulatory T cells (Treg), and downregulate the production of Th1 inflammatory cells.[1-3] A pair of interacting co-inhibitory proteins, B7-H1 (also known as PD-L1) and PD-1, appear to be critical molecules in this process.[3]

Available data in humans support the hypothesis that VitD3 deficiency promotes a breakdown of B7-H1/PD-1 interactions between tolerogenic DCs and Tr1 cells. VitD3 stimulates Treg development, improves IL-10 production and Treg suppressive activity, and inhibits Th1 cell proliferation and inflammatory cytokine production *in vitro*.[4-6] In blood or cerebrospinal fluid from MS patients during an acute phase of the disease, significantly fewer $B7\text{-}H1^+IL\text{-}10^+$ DCs, and reduced PD-1 expression on myelin basic protein antigen-specific T cells were found compared to samples from individuals with a stable form of the disease.[7] In another study, IL-10 suppressive activity and Tr1 function were significantly impaired in patients with MS compared to control subjects.[8] Several methods have been reported for producing "semi-mature" DCs that can induce Tr1 *in vitro*.[9,10] Intriguingly, methods that include VitD3 treatment result in high levels of B7-H1 expression on DCs, and these DCs promote antigen-specific Tr1-mediated tolerance.[9]

In EAE, it is established that prior treatment with VitD3 can prevent induction of the disease, while VitD3 given after the onset of EAE induces remission.[2] Although a direct connection with VitD3 has not yet been made in the mouse, deletion or down-modulation of B7-H1 or PD-1 downregulates Treg suppressive activity, accelerates the establishment of EAE, and exacerbates its severity.[11,12] A B7-H1-Ig fusion protein alone can direct naïve mouse $CD4^+$ T cells toward a Tr1-like phenotype, enhancing secretion of IL-10 and TGF-β, and suppressing Th1 mixed lymphocyte reactions.[13] Similarly, while $B7\text{-}H1^{-/-}$ DCs minimally convert naïve $CD4^+$ cells to Treg cells, beads coated with the B7-H1 protein enhance Treg production and suppressive activity.[14]

Other lines of evidence linking VitD3 to immune functions are also being pursued. For example, VitD3 appears to regulate expression of the pro-inflammatory molecule osteopontin and specific HLA-DR molecules, such as the HLA-DRB1*1501 allele associated with MS susceptibility.[1] The association of VitD3 with cell types and mediators that are critical to immune suppression supports the idea that VitD3 deficiency may increase the risk of autoimmune disorders, such as MS.

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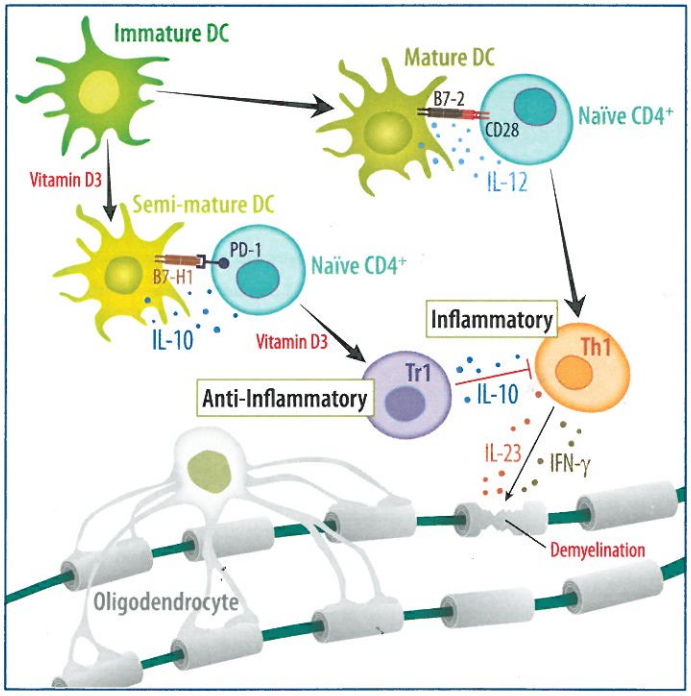

VitD3 Promotes the Differentiation of Immunosuppressive Regulatory T Cells. Upon stimulation of immature DCs, the presence of active VitD3 promotes the formation of tolerogenic, semi-mature DCs (smDCs), rather than mature DCs. These smDCs secrete IL-10 and show increased surface expression of the co-inhibitory molecule, B7-H1, which interacts with PD-1 on the surface of naïve $CD4^+$ T cells. This interaction, aided by the presence of VitD3, promotes antigen-specific Tr1 regulatory T cell differentiation. The Tr1 cells suppress Th1 cell proliferation and production of inflammatory cytokines, such as IFN-γ and IL-23. These and other regulatory T cells modulate the activity of inflammatory antigen-specific effector T cells (such as Th1) in the central nervous system that can promote demyelination. In multiple sclerosis, balance between the anti-inflammatory and inflammatory pathways is compromised.

Developmental Apoptosis & Satellite Glial Cell Precursors

Successful developmental processes require two things: 1) proper timing, and 2) proper positioning. These two factors are, by and large, linked. For example, a decrease in responsiveness to a growth factor may extinguish a mitogenic or chemoattractive effect, while an increase in responsiveness to an environmental cue may have the opposite effect. Cell death can be considered a timing event, one that is widely used during embryogenesis. There are two types of cell death: necrosis and apoptosis. For cell death to be effective during development, it must come in the right form. Necrosis is initiated by external events, characterized by cell rupture, and results in inflammation. In contrast, apoptosis is initiated internally, mediated by caspases, and does not trigger inflammation.[1] Necrotic cell death is often accompanied by scarring, an outcome that may be acceptable in wounded tissue, but is never acceptable in newly created tissue. Apoptosis, if properly regulated, eliminates unwanted cells without damaging the structural integrity of the surrounding tissue. Thus, this form of cell death is highly preferred during development.

Apoptosis during embryogenesis has recently been studied in the mammalian dorsal root ganglia (DRG). During embryonic development, neural crest cells migrate laterally from the superior aspect of the neural trunk. These cells form cellular aggregates that have the potential to generate glia and more than 20 different neuronal cell types.[2] Notably, up to 50% of all cells destined to become neurons will ultimately be removed via apoptosis. The cells responsible for debris removal are satellite glial cell (SGC) precursors.[3] This is somewhat surprising given that cells typically involved in the removal of cellular debris are phagocytes belonging to the macrophage/microglia lineage. In the mouse embryonic DRG, however, Wu *et al.* discovered that SGC precursors were properly placed, sufficiently plentiful, and fully capable of removing dead neuronal cells from embryonic day E11 through E15 (equivalent to day 31 through day 77 in human). Wu *et al.* identified two particular molecules that contribute to the phagocytic process, MEGF10 (multiple EGF domains protein 10), and MEGF12/Jedi-1 (Jagged and Delta-1), a 150 kDa type I transmembrane glycoprotein also known as PEAR-1 in human.[4] Although the ligand(s) for Jedi-1 is unknown, data suggest that MEGF10 and Jedi-1 interact to form a functional complex.

The potential importance of Jedi-1 (and MEGF10) in satellite cell precursor activity is twofold. First, it presumably imparts satellite cells with a phagocytic capacity that meets the rate of neuronal cell death. As long as dying cells retain their cell membrane, pro-inflammatory mediators such as HSP70 and HSP96 remain intracellular, and inflammation is suppressed. But if the apoptotic program ends and secondary necrosis begins (due to an inability to increase the rate of phagocytosis), HSPs are released, leading to macrophage-mediated cytokine secretion and dendritic cell activation.[5] Second, the use of receptors such as Jedi-1 apparently contributes to a tolerogenic form of antigen processing. Wu *et al.* found that inflammation was absent in the DRG.[3] Other glia, such as astrocytes, are reported to remove necrotic neurons in the absence of microglial stimulation. While the data is mixed, astrocytes are reported to be both tolerogenic and immunogenic, depending upon the experimental design.[6,7] Thus, there is a precedent for glial-mediated induction of tolerance. Coupled with this is the observation that once secondary necrosis begins, an aberrant form of antigen processing ensues, exposing antigenic epitopes that otherwise are not detectable. This is suggested to predispose an animal to later autoimmunity, and emphasizes the importance of timely debris removal.[8]

On balance, it would appear that Jedi-1 and its related family members likely play a significant role in the creation of an environment that is both developmentally friendly and homeostatically secure.

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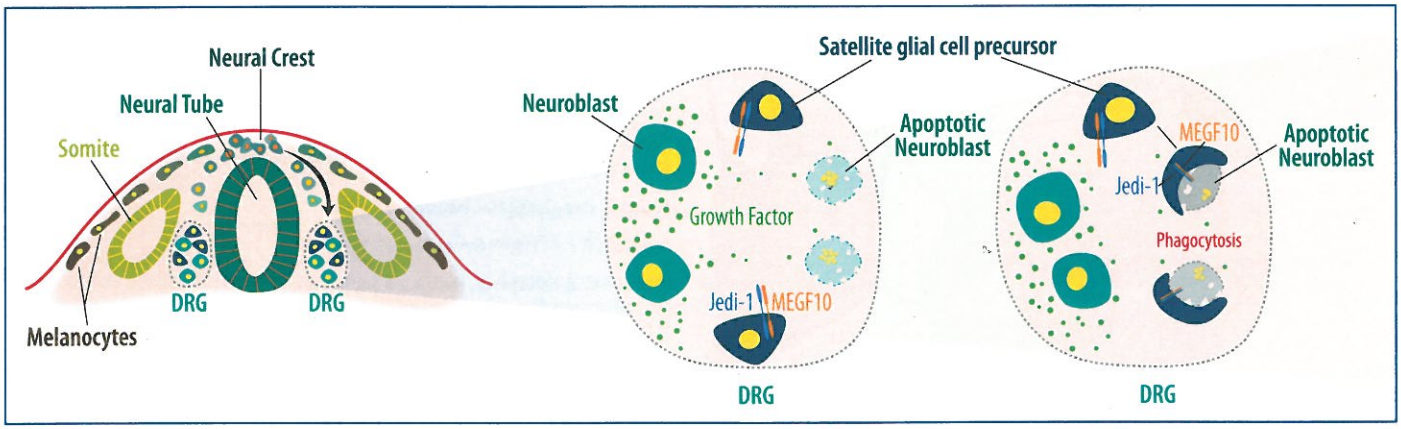


Jedi-1 and MEGF10 on Satellite Glial Cell Precursors Mediate the Phagocytosis of Apoptotic Neurons During Embryonic Development. During embryonic development, neural crest cells migrate ventrolaterally to a position destined to become the dorsal root ganglia (DRG). Some cells become neuroblasts (light green), while others form support cells such as satellite glial cell precursors (blue). Depending upon the neuronal phenotype, specific neurotrophic factors are required for cell survival. Inadequate exposure to a required growth factor initiates an apoptotic program. Apoptotic neuroblasts are cleared via phagocytosis by satellite glial cell precursors. This process involves the transmembrane molecules Jedi-1 and MEGF10.

Wnt Flips a Dual Switch to Activate Adult Neurogenesis

In contrast to a long held belief, the adult mammalian brain is capable of generating new neurons. It is now established that adult neurogenesis occurs in the subventricular zone of the lateral ventricles and the subgranular zone of the hippocampal dentate gyrus. As detection methods improve, it is likely that other areas of the brain will also be considered neurogenic.[1] Studies have shown that a variety of extrinsic factors including exercise, environmental enrichment, and injury stimulate neurogenesis in the adult brain.[2] The current challenge is to determine the signaling pathways that govern this intriguing biological phenomenon.

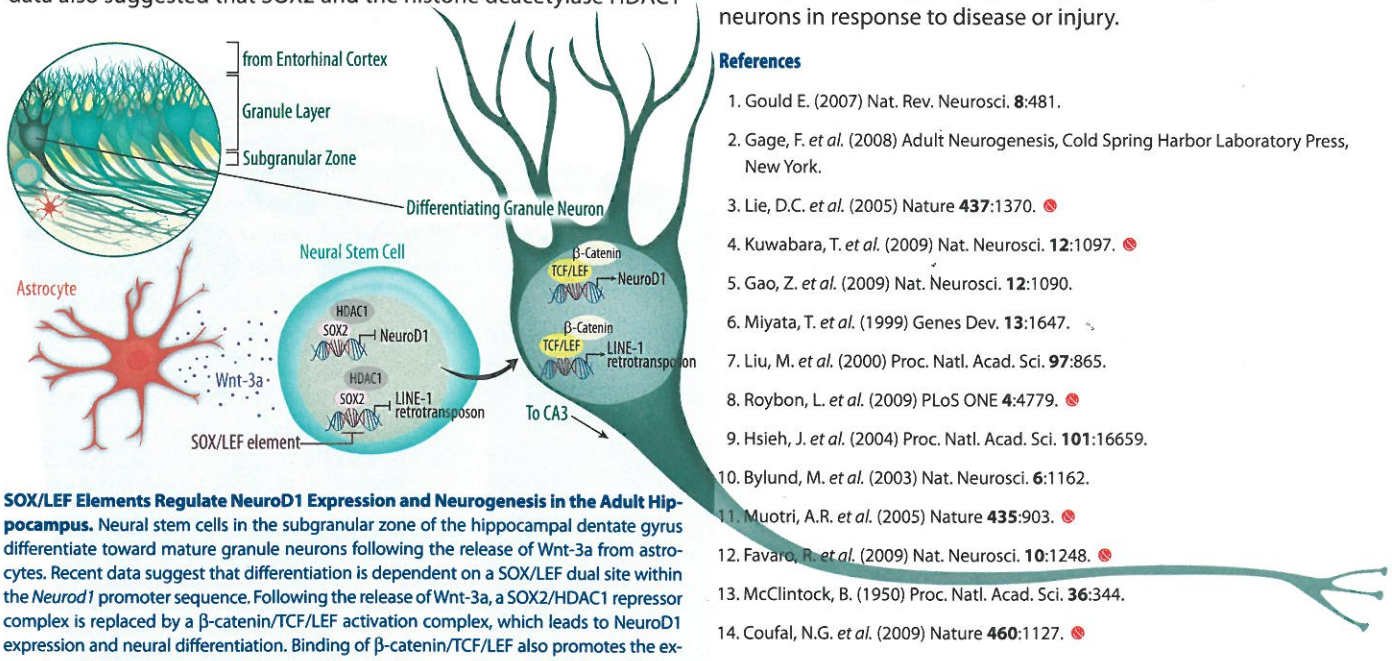
Previous studies showed that astrocytes release Wnt-3 to stimulate adult neurogenesis *in vitro* and *in vivo*, but the underlying molecular mechanism and downstream genetic targets have yet to be identified.[3] Two recent complementary reports focused on the involvement of NeuroD1, a proneural basic helix-loop-helix (bHLH) transcription factor.[4,5] During development, NeuroD1 is critical for the generation of granule neurons in the hippocampus.[6-8] In addition, NeuroD1 overexpression was shown to induce neuronal differentiation in neural progenitor cells isolated from adult rat hippocampus.[9]

To test the hypothesis that Wnt-mediated adult neurogenesis is dependent on NeuroD1, Kuwabara *et al.* studied the *Neurod1* promoter sequence.[4] Using *in silico* analysis, the authors identified a T cell factor/lymphoid enhancer factor (TCF/LEF) regulatory element. TCF/LEF is the major downstream transcription factor that transduces canonical Wnt/β-catenin signaling. Kuwabara and colleagues discovered that this DNA sequence also contains an overlapping binding site for the HMG-box transcription factor, SOX2. This was an intriguing finding because SOX2 is known to prevent neurogenesis during development and is thought to be essential for maintaining neural stem cell populations in neonatal brain.[10-12]

Using reporter constructs, Kuwabara *et al.* conducted experiments in adult rat hippocampal neural stem cells. Their results showed that administration of Wnt-3a upregulated *Neurod1* promoter activity, and this effect was dependent on a functional TCF/LEF binding site. The data also suggested that SOX2 and the histone deacetylase HDAC1 repressor protein associate with the *Neurod1* promoter in undifferentiated cells. Following incubation of the cells with Wnt-3a, the SOX2/HDAC1 repressor complex was replaced by a β-catenin/TCF/LEF activation complex, which induced NeuroD1 expression and neuronal differentiation. Collectively, these *in vitro* findings support the presence of dual regulatory elements (SOX/LEF sites) in the *Neurod1* promoter that bi-directionally regulate adult neurogenesis in the hippocampus. This hypothesis was supported by a series of immunohistochemical, small interfering RNA (siRNA), and chromatin immunoprecipitation (ChIP) experiments.

To investigate the functional relevance of these *in vitro* findings, a complementary study designed *in vivo* experiments using a conditional genetic disruption model. Knock out of the *Neurod1* gene results in mice that develop neonatal diabetes and die perinatally. To address this technical challenge, Gao *et al.* generated *Neurod1* conditional knock out mice (NeuroD1 cKO) in which NeuroD1 expression was specifically ablated in the subgranular zone of the dentate gyrus in the hippocampus.[5] Immunohistochemical analysis revealed no change in the number of total neural stem cells in this neurogenic region following deletion of *Neurod1*, suggesting that NeuroD1 is not required for the formation of early stem or progenitor cells. However, there was a selective loss of newborn granule neurons in NeuroD1 cKO mice, further supporting the hypothesis that NeuroD1 is functionally required for the formation of late stage progenitors and their differentiation into hippocampal granule neurons.

Additional studies by Kuwabara *et al.* found dual SOX/LEF sites in the Long Interspersed Element-1 (LINE-1) retrotransposon.[4] Originally described as "jumping genes", retrotransposons are sequences of DNA that can replicate and reinsert into the genome at different positions.[13] Although their physiological significance remains unclear, these elements are known to upregulate and retrotranspose during neurogenesis.[14] Collectively, these recent studies suggest that dual SOX/LEF sites may represent a molecular switch that couples neural generation and diversification. Their discovery may present a novel pharmacological target to promote the generation of new neurons in response to disease or injury.



SOX/LEF Elements Regulate NeuroD1 Expression and Neurogenesis in the Adult Hippocampus. Neural stem cells in the subgranular zone of the hippocampal dentate gyrus differentiate toward mature granule neurons following the release of Wnt-3a from astrocytes. Recent data suggest that differentiation is dependent on a SOX/LEF dual site within the *Neurod1* promoter sequence. Following the release of Wnt-3a, a SOX2/HDAC1 repressor complex is replaced by a β-catenin/TCF/LEF activation complex, which leads to NeuroD1 expression and neural differentiation. Binding of β-catenin/TCF/LEF also promotes the expression of LINE-1, a retrotransposon thought to be important for neuronal diversification.

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RECENT CITATIONS: R&D Systems Products for Wnt-related Research

1. Gogolla, N. *et al.* (2009) Wnt signaling mediates experience-related regulation of synapse numbers and mossy fiber connectivities in the adult hippocampus. Neuron **62**:510.

   Recombinant Human Wnt-7a (Catalog # 3008-WN)

   Recombinant Mouse Wnt-5a (Catalog # 645-WN)

   Recombinant Mouse Wnt-3a (Catalog # 1324-WN)

   Recombinant Human sFRP-1 (Catalog # 1384-SF)

   Sample: Mouse
   Application: *In Vivo*

   Human Wnt-7b Monoclonal Antibody (Catalog # MAB3460)

   Human Wnt-7a Polyclonal Antibody (Catalog # AF3008)

   Human Wnt-7b Polyclonal Antibody (Catalog # AF3460)

   Human sFRP-1 Polyclonal Antibody (Catalog # AF1384)

   Sample: Mouse brain
   Application: Immunohistochemistry and Western blot

2. Vijayaragavan, K. *et al.* (2009) Noncanonical Wnt signaling orchestrates early developmental events toward hematopoietic cell fate from human embryonic stem cells. Cell Stem Cell **4**: 248.

   Human E-Cadherin Allophycocyanin-labeled Monoclonal Antibody (Catalog # FAB18381A)

   Human/Mouse Brachyury Phycoerythrin-labeled Polyclonal Antibody (Catalog # IC2085P)

   Human/Mouse Frizzled-7 Monoclonal Antibody (Catalog # MAB1981)

   Sample: Human hematopoietic stem cells
   Application: Flow cytometry

   Human Brachyury Polyclonal Antibody (Catalog # AF2085)

   Sample: Human embryoid bodies
   Application: Immunocytochemistry

3. Verkaar, F. *et al.* (2009) Stably overexpressed human Frizzled-2 signals through the β-catenin pathway and does not activate Ca^{2+}-mobilization in human embryonic kidney 293 cells. Cell. Signal. **21**:22.

   Mouse Frizzled-2 Monoclonal Antibody (Catalog # MAB1307)

   Sample: HEK 293 cells transfected with human Frizzled-2
   Application: Cell-Based ELISA

   Recombinant Mouse Wnt-3a (Catalog # 1324-WN)

   Recombinant Mouse Wnt-5a (Catalog # 645-WN)

   Sample: HEK 293 cells transfected with human Frizzled-2
   Application: Luciferase and β-lactamase activity assays

4. Heath, D. *et al.* (2009) Inhibiting Dickkopf-1 (Dkk-1) removes suppression of bone formation and prevents the development of osteolytic bone disease in multiple myeloma. J. Bone Miner. Res. **24**:425.

   Mouse Dkk-1 Polyclonal Antibody (Catalog # AF1765)

   Sample: Mouse decalcified bone
   Application: Immunohistochemistry

5. Hu, J. *et al.* (2009) Blockade of Wnt signaling inhibits angiogenesis and tumor growth in hepatocellular carcinoma. Cancer Res. **69**:6951.

   Human WIF-1 Polyclonal Antibody (Catalog # AF134)

   Human sFRP-1 Polyclonal Antibody (Catalog # AF1384)

   Human/Mouse/Rat β-Catenin Polyclonal Antibody (Catalog # AF1329)

   Sample: Human homogenized tumor
   Application: Western blot

   Recombinant Rat VEGF 164 (Catalog # 564-RV)

   Recombinant Mouse IGF-I (Catalog # 791-MG)

   Sample: Mouse endothelial progenitor cells
   Application: Bioassay - Differentiation

6. Lambert, M. *et al.* (2009) Platelet factor 4 regulates megakaryopoiesis through low-density lipoprotein receptor-related protein 1 (LRP1) and megakaryocytes. Blood **114**:2290.

   Human LRP-1 Cluster II Polyclonal Antibody (Catalog # AF2368)

   Sample: Mouse bone marrow progenitor cells
   Application: Neutralization

   Recombinant Human Thrombopoietin (Catalog # 288-TP)

   Sample: Human $CD34^+$ bone marrow-derived cells
   Application: Bioassay- Megakaryopoiesis

   Recombinant Mouse Thrombopoietin (Catalog # 488-TO)

   Sample: Mouse $CD34^+$ bone marrow-derived cells
   Application: Bioassay- Megakaryopoiesis

7. Kawakami, K. *et al.* (2009) Functional significance of Wnt Inhibitory Factor-1 gene in kidney cancer. Cancer Res. **69**:8603.

   Human WIF-1 Monoclonal Antibody (Catalog # MAB134)

   Sample: Human renal cell carcinoma
   Application: Immunohistochemistry

8. Laumanns, I. *et al.* (2009) The noncanonical Wnt pathway is operative in idiopathic pulmonary arterial hypertension. Am. J. Respir. Cell Mol. Biol. **40**:683.

   Mouse Wnt-11 Polyclonal Antibody (Catalog # AF2647)

   Human Dishevelled-1 Polyclonal Antibody (Catalog # AF3316)

   Sample: Human lung
   Application: Immunohistochemistry

9. Ma, L. *et al.* (2009) Establishment of a transitory dorsal-biased window of localized Ca^{2+} signaling in the superficial epithelium following the mid-blastula transition in zebrafish embryos. Dev. Biol. **327**:143.

   Recombinant Human Wnt-7a (Catalog # 3008-WN)

   Recombinant Mouse Wnt-5a (Catalog # 645-WN)

   Recombinant Human FGF basic (Catalog # 233-FB)

   Sample: Zebrafish superficial epithelial cells
   Application: Bioassay – Calcium signaling

10. Borowiak, M. *et al.* (2009) Small molecules efficiently direct endodermal differentiation of mouse and human embryonic stem cells. Cell Stem Cell **4**:348.

    Human SOX17 Polyclonal Antibody (Catalog # AF1924)

    Sample: Human and mouse embryonic stem cells
    Application: Immunohistochemistry and Flow Cytometry

    Recombinant Human/Mouse/Rat Activin A (Catalog # 338-AC)

    Recombinant Mouse Wnt-3a (Catalog # 1324-WN)

    Recombinant Mouse Nodal (Catalog # 1315-ND)

    Recombinant Human FGF-10 (Catalog # 345-FG)

    Sample: Human and mouse embryonic stem cells
    Application: Bioassay – Growth and differentiation

R&D SYSTEMS REPRESENTATIVES WILL BE AVAILABLE AT THE FOLLOWING CONFERENCES:

International Society for Stem Cell Research Booth # 916
San Francisco, CA.....June 16-19, 2010

Society for Developmental Biology
Albuquerque, NM .....August 5-9, 2010

TECHNICAL NOTE:
R&D Systems MagCellect™ Human & Mouse Natural Killer Cell Isolation Kits

Natural killer (NK) cells are lymphocytes of the innate immune system that function as both cytolytic effectors and regulators of immune responses. NK cells develop from hematopoietic stem cells primarily in the bone marrow and are educated to become self-tolerant by recognition of self MHC class I molecules. Mature NK cells migrate to the liver and peripheral lymphoid organs, including the spleen and lymph nodes. They are activated upon detection of abnormalities in target cells including loss or down-regulation of MHC class I expression, or upregulation of stress-induced ligands that occurs in response to infection or neoplastic transformation.

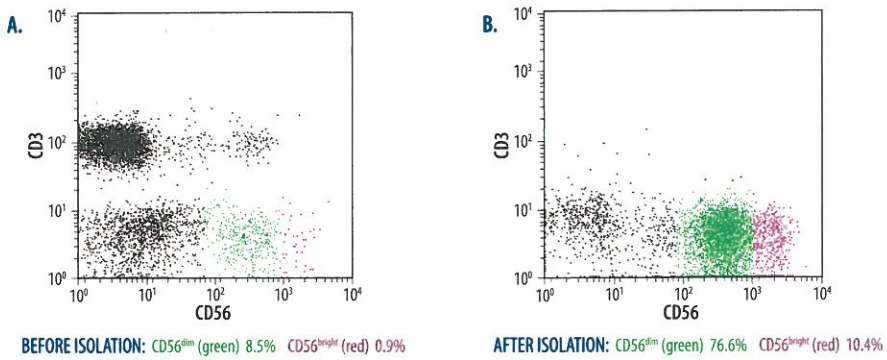
R&D Systems has developed two MagCellect Kits for mouse and human NK cell selection. Both kits are designed to isolate NK cells using a negative selection protocol to remove undesired cells from a mononuclear cell suspension. Unlike positive selection kits, the MagCellect Kits leave the NK cell populations untouched, reducing the risk that the cells will be altered by the selection process. The typical purity of the recovered NK cells ranges between 70-80% for the mouse kit and 80-90% for the human kit.

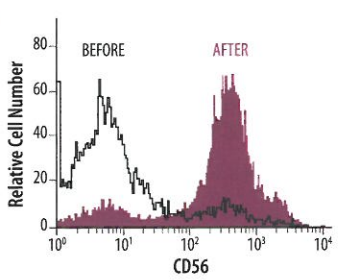
The effectiveness of the negative selection protocol for NK cell isolation was determined by characterizing the phenotypes and the functional capabilities of the cells following isolation. These experiments demonstrated that the isolated cells express a variety of NK cell-specific markers including CD56, NKp46, NKp80, NKp30, NKG2D, KIR3DL1, and NTB-A in human, and NKp46, NKG2D, and CD49b in mouse (see data sheets online). In addition, cells isolated with the human NK Cell Isolation Kit were shown to undergo target cell-induced degranulation (middle panel), and to express NKp46 and Granzyme B by immunohistochemisty (bottom panel). These experiments confirmed that NK cells isolated with the MagCellect Kits express the appropriate markers and remain biologically active.

MagCellect Kit	Catalog #
Human Natural Killer Cell Isolation Kit	MAGH109
Mouse Natural Killer Cell Isolation Kit	MAGM210

For more information on these kits visit our website at **www.RnDSystems.com/go/CellSelection** or **www.RnDSystems.com/go/NKCells** for other products related to NK cell research.

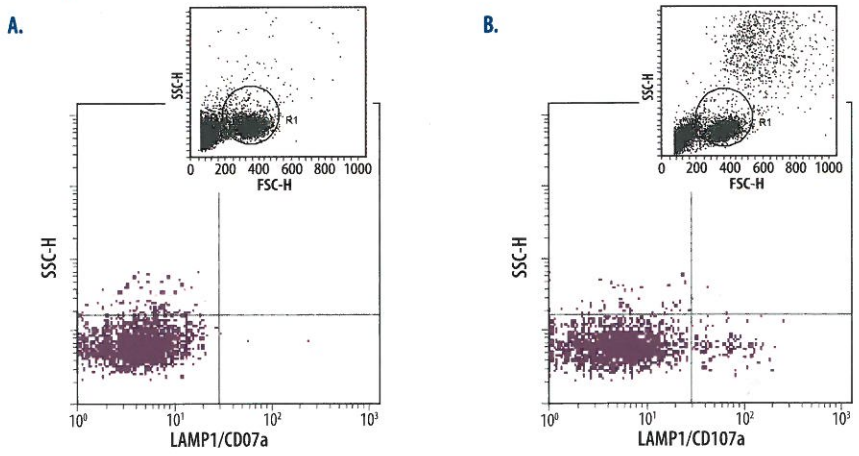
Isolation of Human NK Cells



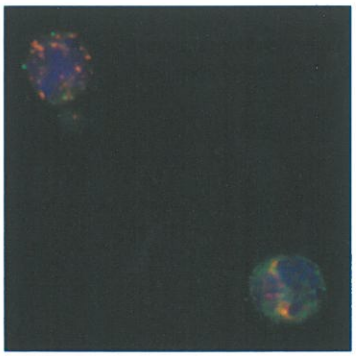


Enrichment of NK Cells from Peripheral Blood Mononuclear Cells using the MagCellect Human NK Cell Isolation Kit. The MagCellect Human NK Cell Isolation Kit (Catalog # MAGH109) was used to enrich for NK cells from a peripheral blood mononuclear cell suspension. Cells were double-stained with APC-conjugated anti-human CD3 monoclonal antibody (Catalog # FAB100A) and PE-conjugated anti-human NCAM-1/CD56 monoclonal antibody (Catalog # FAB2408P) before (A) and after (B) enrichment. CD56dim cells are shown in green and CD56bright cells are shown in red. (C) Corresponding histograms of the CD56 staining before and after selection are shown.

Assessing Cellular Function Following Isolation



Detection of Target Cell-induced NK Cell Degranulation following Isolation using the MagCellect Human NK Cell Isolation Kit. Human peripheral blood natural killer (NK) cells were isolated using the MagCellect Human NK Cell Isolation Kit (Catalog # MAGH109). Isolated cells were incubated alone (A), or with K562 human erythroleukemia cells (B), at an effector to target ratio of 2:1 for 3 hours. NK cell degranulation, as indicated by translocation of LAMP1/CD107a to the cell membrane, was analyzed using APC-conjugated anti-human LAMP1/CD107a monoclonal antibody (Catalog # IC4800A).



Detection of NKp46 and Granzyme B Expression in Human NK Cells Isolated using the MagCellect Human NK Cell Isolation Kit. Human peripheral blood natural killer (NK) cells were isolated using the MagCellect Human NK Cell Isolation Kit (Catalog # MAGH109). NKp46 was detected in isolated cells using anti-human NKp46 polyclonal antibody (Catalog # AF1850) followed by staining with NorthernLights™ 493-conjugated anti-goat secondary antibody (Catalog # NL003; green). Granzyme B was detected using anti-human Granzyme B monoclonal antibody (Catalog # MAB2906) followed by staining with NorthernLights 557-conjugated anti-mouse secondary antibody (Catalog # NL007; red). Nuclei were counterstained with DAPI (blue).

IL-27 continued from page 1

Recent reports have provided more details on the mechanisms by which IL-27 negatively regulates Th17 differentiation and inflammation. Using human or mouse naïve CD4+ T cells cultured under Th17-inducing conditions, IL-27 was shown to inhibit expression of the Th17-specific transcription factor, RORγt, and subsequent secretion of IL-17A.[10] Consistent with published results, IL-27 also induced IL-10 production, suggesting a second mechanism by which it may regulate the pathogenicity of Th17 cells.[10-12] To test the *in vivo* function of IL-27, mice lacking the p28 subunit of IL-27 were generated and immunized with myelin oligodendrocyte glycoprotein to induce experimental autoimmune encephalomyelitis (EAE), a mouse model of multiple sclerosis.[10] Similar to TCCR/WSX-1-deficient mice, IL-27 p28-deficient mice were more susceptible to EAE and developed a significantly more severe form of the disease. The increase in disease severity was associated with elevated expression of Th17-related molecules in the central nervous system, and reduced late phase expression of IL-10.

These results were confirmed in part by Murugaiyan *et al.*, who also found that IL-27 could induce the production of IL-10 and IFN-γ, and inhibit IL-17 secretion by anti-CD3, anti-CD28-activated human CD4+ T cells.[13] This was accompanied by reduced expression of GATA-3 and RORC. Addition of IL-2 to activated T cells significantly enhanced IL-27-induced IL-10 secretion, while a neutralizing antibody to IL-2 inhibited IL-10 production. These characteristics were reminiscent of the phenotype of Tr1 cells, a subset of $CD4^+FoxP3^{+/-}$ $IL\text{-}10^+$ T regulatory (Treg) cells that expand in the presence of IL-2, suggesting that IL-27 may in part confer a Tr1-like activity on CD4+ T cells.[14] Supporting this hypothesis, the supernatants from activated, IL-27-treated T cells suppressed the proliferation of freshly purified CD4+ T cells in an IL-10-dependent manner.[13] Collectively, these studies highlight the pivotal role that IL-27 plays in regulating the delicate balance between pro-inflammatory Th1/Th17 cells and anti-inflammatory IL-10-producing T cell populations.

Crabe *et al.* have also recently described another secreted complex that consists of the p28 subunit of IL-27 and cytokine-like factor 1 (CLF).[15] Like IL-27, p28/CLF is secreted by activated dendritic cells, but it requires TCCR/WSX-1, gp130, and IL-6 Rα for signaling. In contrast to IL-27, p28/CLF not only inhibited the proliferation of mouse naïve CD4+ T cells, but it also induced the expression of IL-17 in the presence of TGF-β. The level of IL-17 expression was comparable to that induced by TGF-β and IL-6, when followed by PMA/ionomycin re-stimulation, demonstrating that p28/CLF could substitute for IL-6 in promoting mouse Th17 differentiation. IL-27 suppressed IL-17 expression in both circumstances, suggesting that it acts as an antagonist of both p28/CLF and IL-6 under these conditions. Further studies are necessary to determine the *in vivo* significance of p28/CLF, and whether this complex is relevant in humans. However, initial *in vitro* characterization indicates that it too may be involved in the regulation of inflammation and autoimmune diseases.

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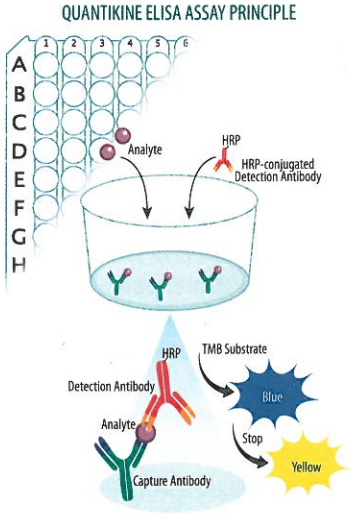

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Clusterin	H	DCLU00	S P C U V
Dkk-1	H	DKK100	S P C
EGF R/ErbB1	H	DEGFR0	S P C M
EG-VEGF/PK1	H	DEGVF0	S P C
FGF-21	M	MF2100	S P C
Galectin-3	H	DGAL30	S P C
GAS6	H	DGAS60	S P C U
GDF-15	H	DGD150	S P C U
IL-17A/F Heterodimer	M	M17AF0	S P C
IL-19	H	D1900	S P C U V M
Lipocalin-2/NGAL	M	MLCN20	S P C U
MBL	H	DMBL00	S P C
Proprotein Convertase 9/PCSK9	H	DPC900	S P C L
Proprotein Convertase 9/PCSK9	M	MPC900	S P C L
Periostin/OSF-2	M	MOSF20	S P C
Progranulin	H	DPGRN0	S P C U V M
ST2/IL-1 R4	H	DST200	S P C
Thrombomodulin/CD141	H	DTHBD0	S P C U L
Tie-1	H	DTE100	S P C L
TIM-1/KIM-1	R	RKM00	S P C U T

SPECIES KEY: **H** Human **M** Mouse
SAMPLE TYPE KEY: **S** Serum **P** Plasma **C** Cell Culture Supernatant **U** Urine **V** Saliva **L** Cell Lysate **M** Human Milk **T** Tissue Homogenate