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R&D Systems Cytokine Bulletin
2010 | Issue 1

cytokine BULLETIN

INSIDE

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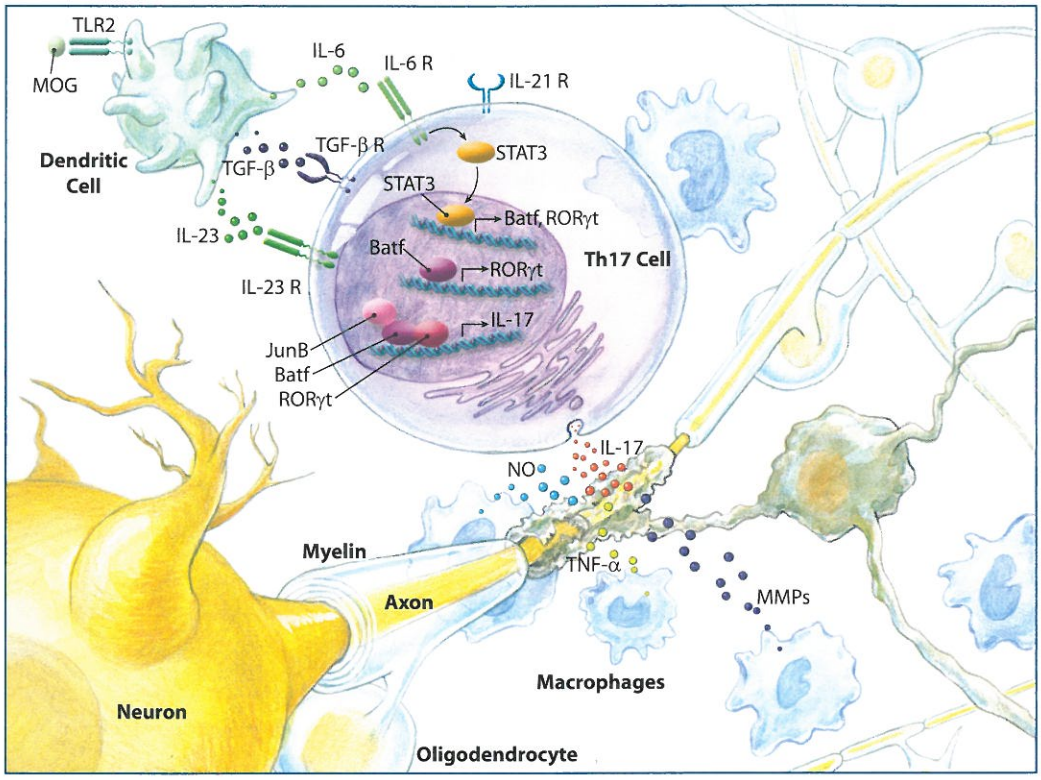

Prolonged production of IL-17 by Th17 cells is dependent on the transcription factor Batf. Recent studies suggest that prolonged production of IL-17 by Th17 cells is dependent on the synergistic actions of the RORγt and Batf-JunB transcription factors. These findings may have implications for Th17-related autoimmune disorders such as multiple sclerosis. Following induction of autoimmune conditions in mice using myelin oligodendrocyte glycoprotein (MOG) immunization, differentiated Th17 cells secrete proinflammatory cytokines, and activated macrophages destroy myelin and damage oligodendrocytes. It remains to be determined whether the induction of Batf expression is dependent on STAT3 in Th17 cells, and whether an interaction between Batf and Irf4 or Ahr is required to promote the respective induction of IL-21 and IL-22.

Th17 Differentiation: An Evolving Target for Multiple Sclerosis Therapy

A growing body of evidence suggests a pathogenic role for T helper 17 cells (Th17) in several autoimmune diseases, including multiple sclerosis (MS). Th17 cells differentiate from naïve $CD4^+$ lymphocytes in the presence of IL-6 and TGF-β.[1] Differentiation of Th17 cells also requires IL-21 for amplification of Th17 precursors, and IL-23 for stabilization and terminal differentiation of Th17 cell populations.[2,3] Differentiated Th17 cells are characterized by the production of proinflammatory cytokines including IL-17A (IL-17), IL-17F, IL-21, and IL-22. It is generally accepted that the STAT3, RORγt, and RORα transcription factors are essential for Th17 cell differentiation, but how their activities are regulated is not completely understood.

A recent study by Schraml and colleagues investigated the contribution of the AP-1 B cell-activating transcription factor (Batf) to T cell differentiation.[4] In these studies, $Batf^{-/-}$ mice were shown to have normally differentiated Th1 and Th2 cells, but lacked differentiated Th17 cells. When stimulated toward a Th17 lineage *in vitro*, $Batf^{-/-}$ T cells produced normal levels of IL-2, IFN-γ, and IL-10, but significantly reduced levels of IL-17. To test the hypothesis that Batf is a critical factor for IL-17 driven autoimmune disease, the authors attempted to induce experimental autoimmune encephalomyelitis (EAE) in $Batf^{-/-}$ mice. EAE is a mouse model of multiple sclerosis in which demyelinating autoimmune conditions are induced following immunization with myelin oligodendrocyte glycoprotein (MOG). Consistent with an underlying pathogenic role for IL-17, $Batf^{-/-}$ mice were completely resistant to the development of EAE.

Continued on page 7

The Cardiovascular Effects of Midkine

Midkine is a heparin-binding growth factor with close structural and functional similarity to Pleiotrophin. Both of these proteins are involved in nervous system development as well as inflammation and the response to ischemia. Midkine and Pleiotrophin are upregulated in many cancers where they promote multiple aspects of tumor growth.[1] Conversely, Midkine is also known to block VEGF-induced proliferation of vascular endothelial cells providing an anti-tumor effect.[2] Additional functions for Midkine in the cardiovascular system have recently been described that expand its range of impact.

The Renin-Angiotensin System (RAS) is critically important for maintaining the volume and electrolyte balance of the blood. It is initiated by secretion of the protease Renin from renal juxtaglomerular cells. Renin cleaves Angiotensinogen to release the peptide Angiotensin I (Ang I); Ang I is subsequently trimmed by Angiotensin I-converting enzyme (ACE) to produce Angiotensin II (Ang II). Ang II signaling through the AT_1 receptor induces a rise in blood pressure via vasoconstriction, and increases heart contractility, aldosterone release, and sodium retention in the kidney.

Midkine contributes to the activation of the RAS. In an experimental model known as 5/6 nephrectomy, renal failure is simulated by removal of one kidney and infarction of 2/3 of the second kidney. This triggers activation of the RAS in lung and brain tissue resulting in systemic hypertension.[3,4] Circulating levels of Midkine rise due to increased production in the lung and remnant kidney vascular epithelium, and this plays an integral role in the hypertensive response.[3] Midkine expression may be induced by oxidative stress since 5/6 nephrectomy also stimulates the production of NADPH oxidase-1, -2, and -4 in the lung leading to the presence of more reactive oxygen species.[3] In Midkine-deficient mice that undergo 5/6 nephrectomy, ACE induction in the lung and the hypertensive response are absent, and subcutaneous administration of Midkine restores both.[3] Knockout of Midkine (in the absence of renal failure) modulates the expression of several RAS components in the aorta as well.[5]

Midkine also assists in tissue recovery from myocardial infarction (MI) by limiting the progression of cardiac remodeling. Remodeling, which ultimately results in heart failure, is characterized by a loss of functional muscle mass in the infarcted area, cardiomyocyte (CM) hypertrophy in the noninfarcted area, and tissue fibrosis due to increased collagen deposition.[6] Midkine expression is transiently induced in the heart following MI, preferentially by CM bordering the infarcted region.[7-9] Exogenous Midkine induces a number of favorable local responses that are otherwise absent in Midkine-deficient animals.[7-9] These include increases in the amount of viable cardiac muscle and angiogenesis in the infarcted area, and decreases in ischemic cell apoptosis, CM hypertrophy, and collagen deposition. These effects improve heart function and survival.[7,8] Pleiotrophin is also upregulated in the heart following myocardial infarction, although in contrast to Midkine, it induces CM apoptosis *in vitro*.[10]

Elevated blood pressure is generally considered to be a risk factor for myocardial infarction. It is therefore intriguing how Midkine can contribute to the hypertensive response in renal failure, and at the same time, play a protective role in preventing heart failure. An explanation may lie in the involvement of distinct Ang II receptors. The AT_2 receptor is primarily expressed during development, but is upregulated in injured heart tissue. AT_2 appears to mediate the cardioprotective functions of Ang II, while AT_1 carries out most of the hypertension-related actions of Ang II.[11] Midkine is required for Ang II production in both settings, with the opposing outcomes potentially being due to Ang II function rather than Midkine itself.

References

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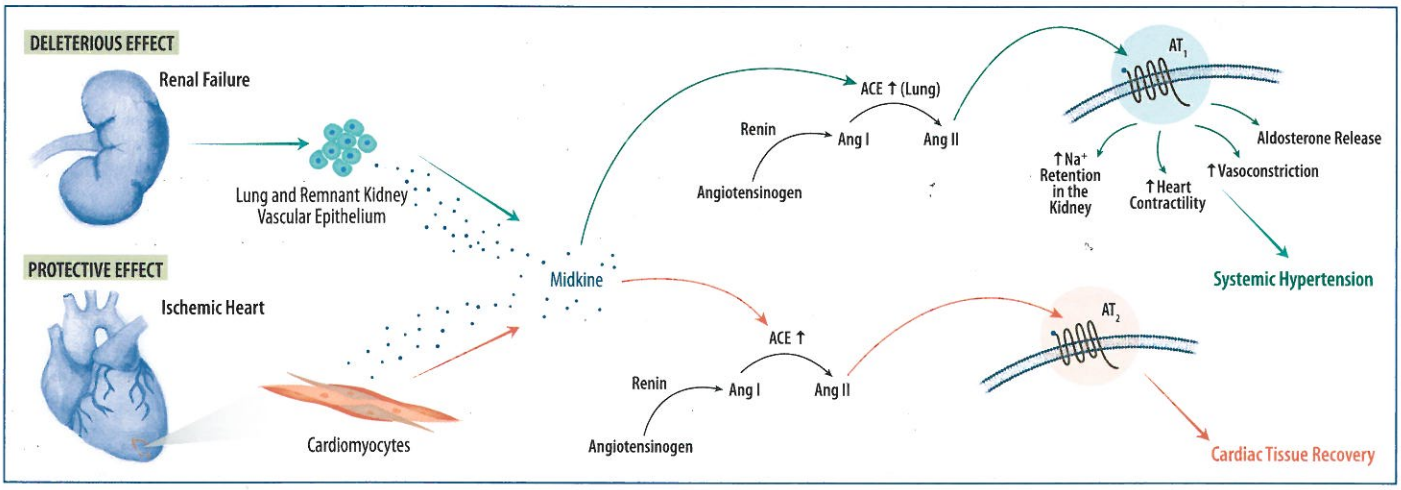


Dual Actions of Midkine on the Cardiovascular System. Midkine can have either deleterious or protective effects on the cardiovascular system. Loss of renal function induces Midkine-dependent upregulation of ACE in the lung, which promotes systemic hypertension. In contrast, Midkine is also upregulated in ischemic heart tissue where it slows cardiac remodeling and promotes the recovery of heart function. While Midkine promotes Ang II production in both situations, the difference in the responses may be determined by whether Ang II subsequently binds to the AT_1 or AT_2 receptor.

Proinflammatory Cytokines & CCR2 Promote Estrogen-deficient Bone Loss

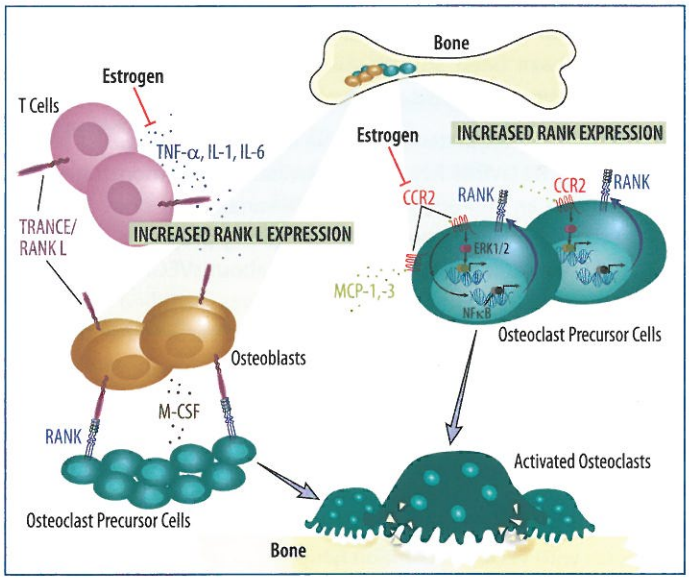
Osteoporosis is an age-related skeletal disease characterized by a rapid and sustained loss of bone density, and an increased susceptibility to bone fractures. The leading cause of osteoporosis in women is a reduction in estrogen production following menopause.[1] Estrogen deficiency is associated with a disruption of bone homeostasis caused by an increase in the number and activity of bone-resorbing osteoclasts. Under normal physiological conditions, bone structure is maintained by a coupling of osteoclast activity with the activity of bone-forming osteoblasts. Osteoblasts regulate osteoclast differentiation through the expression of RANK L and M-CSF. RANK L binding to RANK on osteoclast precursor cells promotes their differentiation. Factors that affect this process stimulate changes in bone mass that can lead to a variety of bone disorders. While the connection between estrogen deficiency and osteoporosis has been known for some time, cellular factors responsible for this effect are still being elucidated.

Proinflammatory cytokines are one critical group of proteins that contribute to the bone-wasting effects associated with estrogen deficiency. Studies in ovariectomized mice, a model of postmenopausal osteoporosis, demonstrated that estrogen deficiency leads to an up-regulation of IL-1, IL-6, M-CSF, and TNF-α, which enhances bone resorption in part by increasing the pool of preosteoclasts in the bone marrow.[1] TNF-α, in particular, plays a central role. It not only induces the expression of RANK L on osteoblasts and stromal cells, but is also involved in regulating the production of other osteoclastogenic cytokines.[2-6] Increased T cell activity largely accounts for the high levels of TNF-α associated with estrogen deficiency.[3,5,7] Significantly, both TNF-deficient and T cell-deficient mice are protected from ovariectomy-induced bone loss.[3,5] While adoptive transfer of wild-type T cells into T cell-deficient mice re-establishes estrogen-deficient bone loss, transfer of T cells from TNF-deficient mice does not.[5] Although bone metabolism may differ significantly in mice and humans, strong evidence suggests that increased T cell expression of TNF-α and RANK L is involved in promoting estrogen-deficient bone loss in humans as well.[8-10]

C-C chemokine receptor 2 (CCR2) is another factor that was recently demonstrated to be involved in mediating bone loss in the absence of estrogen.[11] CCR2 is a G protein-coupled receptor that is activated by chemokines such as monocyte chemoattractant protein-1 and -3 (MCP-1 and MCP-3). Both CCR2 and MCP-1 were previously shown to be induced during osteoclast differentiation, but little was known about the effects of CCR2-dependent signaling on bone loss.[12-14] Using CCR2-deficient mice, Binder *et al.* discovered that there is a significant increase in bone mass and stability in the absence of CCR2.[11] This increase is attributable to a defect in the differentiation of bone marrow macrophages (BMMs) into mature osteoclasts in $Ccr2^{-/-}$ mice. While wild-type and CCR2-deficient mice had similar numbers of BMMs expressing CD11b, a marker of preosteoclasts, BMMs from CCR2-deficient mice had a lower percentage of RANK-positive cells and reduced expression of RANK-dependent genes. Furthermore, treatment with MCP-1 or MCP-3 induced the expression of RANK in wild-type BMMs, but had no effect on CCR2-deficient BMMs. These results support the involvement of CCR2 in the regulation of RANK-dependent osteoclast differentiation. To determine if CCR2 contributes to bone loss under estrogen-deficient conditions, wild-type and CCR2-deficient mice were examined following ovariectomy.

In wild-type mice, ovariectomy led to an increase in both the number of $CD11b^{high}$ preosteoclasts and the expression of CCR2 and RANK on these cells. In CCR2-deficient mice, there was a similar increase in the number of $CD11b^{high}$ preostoclasts, but a less significant increase in RANK expression. More importantly, lack of CCR2 protected against ovariectomy-induced bone loss, indicating that CCR2 signaling may also contribute to the pathogenesis of postmenopausal osteoporosis.

Collectively, these studies suggest that bone resorption under estrogen-deficient conditions is stimulated by two mechanisms: 1) an elevated number of TNF-α-producing T cells, which promote RANK L-dependent osteoclastogenesis, and 2) an increase in the expression of CCR2 on preosteoclasts, which induces RANK expression and increases the likelihood of osteoclast differentiation.



Two Primary Mechanisms Promote Increased Osteoclastogenesis and Bone Resorption in the Absence of Estrogen. Under estrogen-deficient conditions, T cells produce elevated levels of proinflammatory cytokines including TNF-α, IL-1, and IL-6. These cytokines promote increased RANK L expression on osteoblasts and stromal cells, which leads to osteoclast differentiation in the presence of M-CSF. In addition, CCR2 expression on osteoclast precursor cells is upregulated in the absence of estrogen. CCR2 signaling promotes the expression of RANK on these cells and increases their osteoclastogenic potential. Both mechanisms may contribute to the pathogenesis of postmenopausal osteoporosis.

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Soluble VEGF R2: Controlling Lymphangiogenesis

Under normal physiological conditions, angiogenesis (blood vessel formation) and lymphangiogenesis (lymphatic vessel formation) are tightly regulated processes that occur exclusively during embryonic development, pregnancy, and wound healing. Vascular endothelial growth factor (VEGF) and VEGF receptors are key mediators of this regulation. VEGF-A,-B,-C, and -D, and VEGF receptors 1, 2, and 3 differ in both their expression profiles and their functions. In addition, multiple variants of VEGF ligands and VEGF receptors exist that are produced by alternative splicing or cleavage. VEGF R1 (also called Flt-1) and VEGF R2 (also called Flk-1 or KDR) are angiogenic type I transmembrane receptors that primarily respond to VEGF-A.[1-3] Although the affinity of VEGF-A is highest for VEGF R1, VEGF R2 appears to be the primary mediator of VEGF angiogenic activity.[1] In contrast, lymphatic development is associated with the response of VEGF R3 to VEGF-C. While the effects of VEGF signaling on angiogenesis have been studied extensively, the mechanisms controlling lymphangiogenesis are much less clear.[1-3]

VEGF signaling is regulated by soluble forms of both VEGF R1 (sVEGF R1) and VEGF R2 (sVEGF R2). In 2006, Ambati *et al.* demonstrated that sVEGF R1 was an anti-angiogenic splice variant that since has been shown to play a role in preeclampsia during pregnancy.[3,4] In contrast, relatively little has been discovered about sVEGF R2. sVEGF R2 was first reported by Ebos *et al.* in 2004.[5] It was identified to be a 160 kDa protein in the circulation, but it was not clear whether this form is a proteolytic cleavage product or an alternatively spliced isoform, or whether it is a monomer, a homodimer, or a heterodimer.[5,6] A recent paper by Albuquerque *et al.* sheds new light on the regulation of lymphatic vessel growth by characterizing the expression and function of a 75 kDa soluble form of VEGF R2.[7] This form has surprisingly low affinity for VEGF-A, but instead binds to VEGF-C and competes with VEGF R3 to inhibit lymphangiogenesis.[7]

As an avascular tissue, the cornea of the eye has been widely used to study the control of both angiogenesis and lymphangiogenesis.[2,3] In the adult mouse cornea, sVEGF R2 (75kDa) is found only at the periphery, but it is expressed at a much higher level in newborn mice.[7] Tissue-specific loss of corneal sVEGF R2 (75kDa) expression in newborn mice leads to abundant lymphangiogenesis without corresponding angiogenesis, similar to what is observed if VEGF-C is overexpressed. Treatment of corneal transplants with monomeric sVEGF R2 (75kDa) blocks only lymphangiogenesis, in contrast to soluble dimeric VEGF R2-Fc, which inhibits both lymphatic and blood vessel formation. Both forms are effective in decreasing the frequency of corneal transplant rejection, possibly due to reduced lymphatic communication with the immune system. Albuquerque *et al.* identified a similar activity associated with sVEGF R2 (75kDa) in the human cornea.[7] The human form was also shown to be effective in blocking VEGF-C-mediated proliferation of human lymphangiomas *in vitro*.[7]

Studies performed using exogenous sVEGF R2 or blocking antibodies directed against VEGF R2 have generated conflicting results.[5-10] Albuquerque *et al.* provide a possible explanation. They propose that in contrast to the 75 kDa monomeric form of sVEGF R2, Fc-linked recombinant VEGF R2 and possibly other endogenous dimeric soluble forms, retain the ability to bind to VEGF-A and interfere with angiogenesis.[7] Blocking antibodies to VEGF R2, depending on their recognition site, may therefore affect the transmembrane form, one or more soluble forms, or multiple forms, producing entirely different results depending on which form is inhibited. VEGF R2 antibodies are being evaluated in clinical trials as anti-angiogenic agents for the treatment of breast, prostate, ovarian, renal, skin, and hepatic cancers. These antibodies appear to inhibit tumor angiogenesis as expected, but may be less effective as anti-lymphangiogenic agents.[3,9-11] Since lymphatic vessels allow cancer cell migration to the lymph nodes, it is conceivable that these antibodies could have unintended effects on cancer dissemination.[2]

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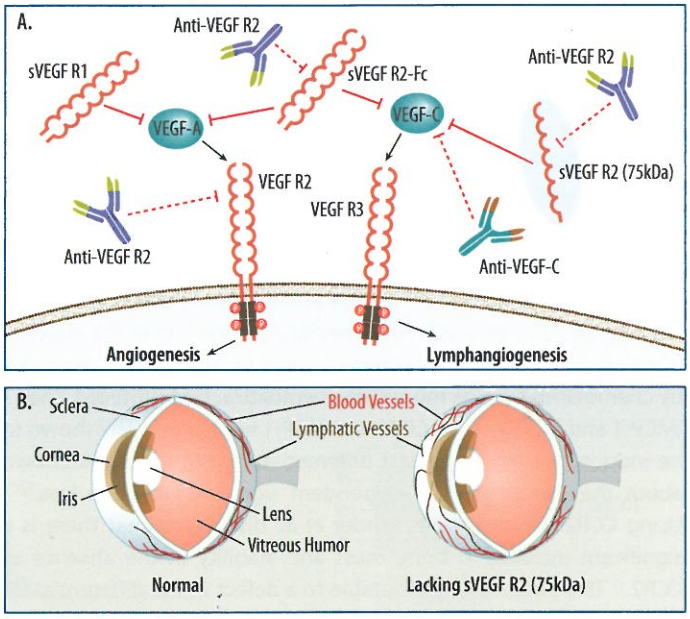


Molecular Mechanisms Regulating Vessel Growth in the Cornea. A. Angiogenesis and lymphangiogenesis are regulated by different forms of VEGF binding to three receptor tyrosine kinases, VEGF R1, VEGF R2, and VEGF R3. VEGF-A binding to VEGF R2 primarily regulates angiogenesis, while VEGF-C binding to VEGF R3 regulates lymphangiogenesis. These processes can be inhibited by soluble forms of the VEGF receptors, and by anti-VEGF or anti-VEGF R antibodies. **B.** The role of a 75 kDa form of sVEGF R2 in lymphangiogenesis has recently been revealed by studying its effects in the cornea of the eye. In the normal cornea, blood and lymphatic vessels are present in the sclera but do not significantly invade the cornea. If sVEGF R2 (75kDa) is not present during development, the newborn cornea is invaded by lymphatic vessels, but not by blood vessels. Lymphatic vessel invasion can be inhibited by either ectopic expression of sVEGF R2 (75kDa) or administration of a VEGF-C antibody. These studies suggest that anti-VEGF R2 antibodies may have variable effects on angiogenesis and lymphangiogenesis depending on which forms of the receptor are recognized by the antibody.

RECENT CITATIONS: R&D Systems Products for Th17-related Research

1. Boniface, K. *et al.* (2009) Prostaglandin E2 regulates Th17 cell differentiation and function through cyclic AMP and EP2/EP4 receptor signaling. J. Exp. Med. **206**:535.

Recombinant Human IL-1β/IL-1F2 (Catalog # 201-LB)

Sample: Mouse T cells, human monocyte/naïve T cell co-culture
Application: Bioassay - Th17 differentiation

Recombinant Human TGF-β1 (Catalog # 240-B)
Recombinant Human IL-6 (Catalog # 206-IL)

Sample: Mouse T cells
Application: Bioassay - Th17 differentiation

Recombinant Human/Rhesus Macaque/Feline CXCL12/SDF-1α (Catalog # 350-NS)
Recombinant Human CCL20/MIP-3α (Catalog # 360-MP)

Sample: Mouse T cells
Application: Chemotaxis

Human IL-23 R Biotinylated Polyclonal Antibody (Catalog # BAF1400)

Sample: Human naïve T cells
Application: Flow cytometry

Human IL-17 Quantikine® ELISA Kit (Catalog # D1700)
Human IL-22 Quantikine ELISA Kit (Catalog # D2200)
Human CCL20/MIP-3α Quantikine ELISA Kit (Catalog # DM3A00)
Human IL-1β/IL-1F2 Quantikine ELISA Kit (Catalog # DLB50)
Human IL-10 Quantikine ELISA Kit (Catalog # D1000B)

Sample: Human T cell culture supernates
Application: ELISA

Mouse IL-17 Quantikine ELISA Kit (Catalog # M1700)

Sample: Mouse T cell culture supernates
Application: ELISA

2. Derfuss, T. *et al.* (2009) Contactin-2/TAG-1-directed autoimmunity is identified in multiple sclerosis patients and mediates gray matter pathology in animals. Proc. Natl. Acad. Sci. U.S.A. **106**:8302.

Recombinant Human Contactin-2/TAG1 (Catalog # 1714-CN)

Sample: Human serum
Application: ELISA - Autoantibody Capture

Human IFN-γ ELISpot Kit (Catalog # EL285)
Human IL-17 ELISpot Kit (Catalog # EL317)

Sample: Human peripheral blood mononuclear cells
Application: ELISpot

3. Ishigame, H. *et al.* (2009) Differential roles of Interleukin-17A and -17F in host defense against mucoepithelial bacterial infection and allergic responses. Immunity **30**:108.

Mouse IL-17F Polyclonal Antibody (Catalog # AF2057)
Mouse IL-17F Biotinylated Polyclonal Antibody (Catalog # BAF2057)

Sample: Mouse colonic lymphocytes
Application: Flow cytometry

Recombinant Mouse IL-17A (Catalog # 421-ML)
Recombinant Mouse IL-17F (Catalog # 2057-IL)

Sample: CMT93 mouse colonic epithelial cell line, peritoneal macrophages, and T cells
Application: Bioassay - stimulation

Mouse IL-17 DuoSet® ELISA Development System (Catalog # DY421)
Mouse IL-17F DuoSet ELISA Development System (Catalog # DY2057)

Sample: CMT93 mouse colonic epithelial cell line, peritoneal macrophages, and T cell culture supernates
Application: ELISA

4. Guttman-Yassky, E. *et al.* (2008) Low expression of the IL-23/Th17 pathway in atopic dermatitis compared to psoriasis. J. Immunol. **181**:7420.

Recombinant Human IL-17A (Catalog # 317-IL)
Recombinant Human IL-22 (Catalog # 782-IL)
Recombinant Human IFN-γ (Catalog # 285-IF)

Sample: Human keratinocytes
Application: Bioassay - gene regulation

Human Lipocalin-2/NGAL Monoclonal Antibody (Catalog # MAB1757)
Human IL-22 Monoclonal Antibody (Catalog # MAB782)
Human IL-23 R Monoclonal Antibody (Catalog # MAB14001)
Human S100A7 Polyclonal Antibody (Catalog # AF4475)
Mouse S100A9 Monoclonal Antibody (Catalog # MAB2065)
Human CCL20/MIP-3α (Catalog # MAB360)

Sample: Human psoriasis and atopic dermatitis skin lesion biopsy
Application: Immunohistochemistry

5. Atarashi, K. *et al.* (2008) ATP drives lamina propria Th17 cell differentiation. Nature **455**:808.

Mouse CCR6 Phycoerythrin-labeled Monoclonal Antibody (Catalog # FAB590P)

Sample: Mouse Th17 cells
Application: Flow cytometry

6. Di Stefano, A. *et al.* (2009) T helper type 17-related cytokine expression is increased in the bronchial mucosa of stable chronic obstructive pulmonary disease patients. Clin. Exp. Immunol. **157**:316.

Human IL-17 Polyclonal Antibody (Catalog # AF-317-NA)
Human IL-17F Polyclonal Antibody (Catalog # AF1335)
Human IL-22 Polyclonal Antibody (Catalog # AF782)

Sample: Human bronchial biopsy
Application: Immunohistochemistry

7. Lemos, H.P. *et al.* (2009) Prostaglandin mediates IL-23/IL-17-induced neutrophil migration in inflammation by inhibiting IL-12 and IFN-gamma production. Proc. Natl. Acad. Sci. U.S.A. **106**:5954.

Recombinant Mouse IL-23 (Catalog # 1887-ML)
Recombinant Mouse IL-17A (Catalog # 421-ML)
Recombinant Mouse IL-12 (Catalog # 419-ML)
Recombinant Mouse IFN-γ (Catalog # 485-MI)

Sample: Mouse
Application: *In vivo*

Recombinant Mouse CXCL2/MIP-2 (Catalog # 452-M2)

Sample: Mouse bone marrow-derived neutrophils
Application: Chemotaxis

Mouse LIX Polyclonal Antibody (Catalog # AF433)
Mouse IL-17 Polyclonal Antibody (Catalog # AF-421-NA)
Mouse CXCL1/KC Polyclonal Antibody (Catalog # AF-453-NA)

Sample: Mouse
Application: *In vivo* neutralization

Mouse IFN-γ Polyclonal Antibody (Catalog # AF-485-NA)
Mouse IL-23 p19 Polyclonal Antibody (Catalog # AF1619)

Sample: Whole mouse, cultured mouse popliteal or inguinal lymph node cells
Application: *In vivo* neutralization, *in vitro* neutralization

Mouse CXCR2 Monoclonal Antibody (Catalog # MAB2164)

Sample: Mouse popliteal or inguinal lymph node cells
Application: Neutralization

Mouse CXCL1/KC Monoclonal Antibody (Catalog # MAB453)
Mouse CXCL1/KC Biotinylated Polyclonal Antibody (Catalog # BAF453)
Mouse IFN-γ Monoclonal Antibody (Catalog # MAB785)
Mouse IFN-γ Biotinylated Polyclonal Antibody (Catalog # BAF485)
Mouse IL-17 Monoclonal Antibody (Catalog # MAB721)
Mouse IL-17 Biotinylated Polyclonal Antibody (Catalog # BAF421)
Mouse IL-12/IL-23 p40 Biotinylated Polyclonal Antibody (Catalog # BAF499)

Sample: Mouse homogenized knee joint tissue
Application: ELISA

TECHNICAL NOTE: R&D Systems
Antibodies & ELISAs for Cell Cycle Checkpoint Research

Cell cycle progression is regulated by a series of checkpoints that monitor the integrity of the DNA. When DNA damage is present, checkpoints are activated to slow or arrest the cell cycle and promote DNA repair or apoptosis. Cell cycle checkpoints rely on sensor proteins that bind to damaged DNA and initiate the recruitment of several other proteins to the damage site. Some of these proteins are responsible for maintaining checkpoint activation as long as DNA damage persists, while others transduce the damage signal by phosphorylating multiple downstream effector proteins. These phosphorylation events directly inhibit the transition from one phase of the cell cycle to the next. Mutations in cell cycle checkpoint proteins are associated with genomic instability syndromes and a predisposition to multiple types of cancer. Research is currently being conducted to reveal the functions of checkpoint proteins and the effects of critical phosphorylation events. This will provide insight into the mechanisms by which checkpoints are regulated and maintained. R&D Systems offers a wide selection of phospho-specific and total protein antibodies and ELISAs for the characterization of cell cycle checkpoint pathways (Table 1). For more information on products for cell cycle- and checkpoint-related research, please visit our website at **www.RnDSystems.com/go/Genotoxic**.

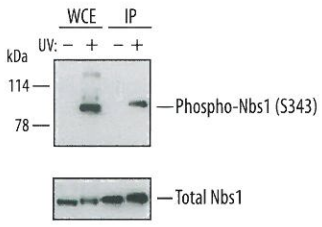
Table 1.

Molecule	Antibodies	Antibody Applications	ELISAs/Kinase Activity Assays
Phospho-53BP1 (S25)	H	WB	
Total 53BP1	H	WB	
Phospho-ATM (S1981)	H M R	WB	H
Total ATR	H	WB	
Total ATRIP	H	WB	
Phospho-BRCA1 (S1423)	H	IHC, WB	
Total BRCA1	H M R	IHC, IP, WB	
Phospho-Chk1 (S317)	H M R	WB	
Phospho-Chk1 (S345)	H	WB	
Total Chk1	H	IP, WB	H/M/R
Phospho-Chk2 (T68)	H	IHC, WB	H
Total Chk2	H M R	IHC, WB	H/M/R
Total Claspin	H	IHC, WB	
Phospho-H2AX (S139)	H	IHC, WB	H/M/R
Total H2AX	H M R	WB	

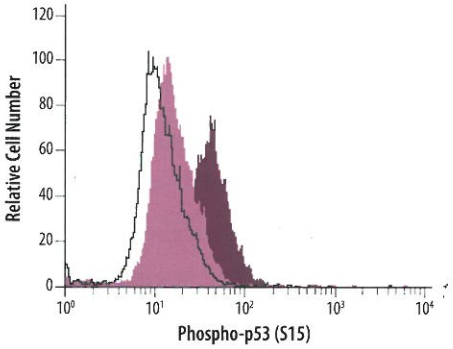
Molecule	Antibodies	Antibody Applications	ELISAs/Kinase Activity Assays
Total Mre11	H	WB	
Phospho-Nbs1 (S343)	H	WB	
Total Nbs1	H M R	IP, WB	
Phospho-p53 (S15)	H	FC, IHC, IP, WB	H
Phospho-p53 (S18)	M	WB	
Phospho-p53 (S20)	H	WB	
Phospho-p53 (S37)	H	WB	
Phospho-p53 (S46)	H	IHC, WB	H
Phospho-p53 (S392)	H	WB	H
Total p53	H M R	FC, IP, WB	H M
Phospho-Rad17 (S635)	H	WB	H
Total Rad17	H M R	IP, WB	H
Total Rad50	H	WB	
Phospho-SMC1 (S966)	H	WB	
Total SMC1	H M R	WB	

Species Key: H Human M Mouse R Rat

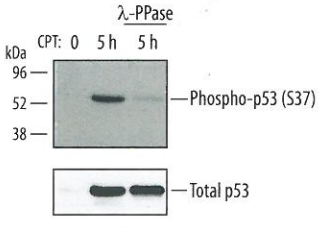
Antibody Application Key: FC Flow Cytometry IP Immunoprecipitation IHC Immunohistochemistry WB Western Blot



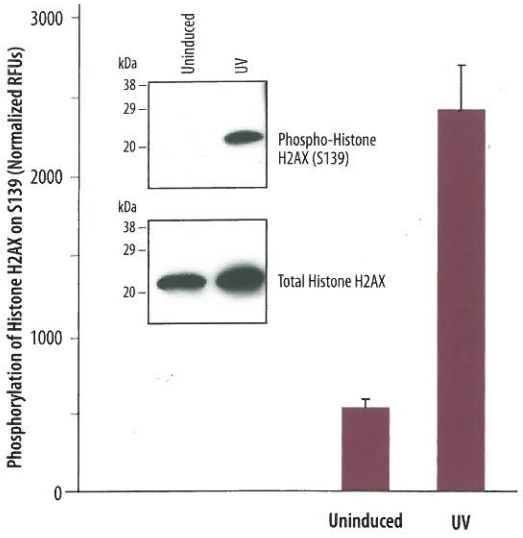
Detection of Phosphorylated Nbs1 on S343 by Western Blot. Exponentially growing HeLa human cervical epithelial carcinoma cells were mock-treated or treated with UV light. Nbs1 was immunoprecipitated from whole cell extracts prepared 3 hours after irradiation using anti-human Nbs1 polyclonal antibody (Catalog # AF1573) and Protein G agarose beads. Following elution, immunoprecipitates (IP) were immunoblotted alongside whole cell extracts (WCE) using anti-human Phospho-Nbs1 (S343) polyclonal antibody (Catalog # AF4944; top panel). The same membrane was stripped and reprobed with anti-human/mouse/rat Nbs1 monoclonal antibody (Catalog # MAB1573; bottom panel)



Detection of Phosphorylated p53 on S15 by Flow Cytometry. MCF-7 human breast cancer cells, untreated (light burgundy histogram) or treated with camptothecin (burgundy histogram), were incubated with anti-human Phospho-p53 (S15) monoclonal antibody (Catalog # MAB18391) or isotope control antibody (Catalog # MAB002; open histogram) followed by staining with PE-conjugated anti-mouse IgG (Catalog # F0102B).



Detection of Phosphorylated p53 on S37 by Western Blot. MCF-7 human breast cancer cells were mock-treated or treated with camptothecin (CPT) for 5 hours. Whole cell extracts were prepared and the indicated sample was treated with λ phosphatase (λ-PPase). p53 was immunoprecipitated from the extracts using anti-human/mouse/rat p53 agarose (Catalog # GAF1355). Following elution, immunoprecipitated proteins were immunoblotted using anti-human Phospho-p53 (S37) polyclonal antibody (Catalog # AF3306; top panel). The same membrane was stripped and reprobed with HRP-conjugated anti-human/mouse/rat p53 polyclonal antibody (Catalog # HAF1355; bottom panel).



Detection of UV-induced Histone H2AX Phosphorylation on S139 using the Cell-based ELISA. HEK293 human embryonic kidney cells were seeded in a 96-well plate and either left untreated or treated with UV light. Following fixation and permeabilization of the cells, phosphorylation of Histone H2AX on S139 was determined using the human/mouse/rat Phospho-Histone H2AX (S139) Cell-Based ELISA (Catalog # KCB2288) and normalized to total Histone H2AX in the same wells (bar graph). Values represent the mean ± the range of duplicate determinations. Cell-based ELISAs allow the simultaneous detection of two proteins in the same microplate well without the need for lysate preparation. Detection of Histone H2AX phosphorylation on S139 by Western blot is shown for comparison (inset).

Th17 continued from page 1

A previous study found that IL-6 knockout mice were resistant to the development of EAE due to a compensatory increase in anti-inflammatory FOXP3$^+$ T regulatory (T_{reg}) cells.[5] However, no changes in FOXP3 expression were observed following immunization with MOG in Batf$^{-/-}$ mice, suggesting that the protective effect associated with a loss of Batf was not dependent on a compensatory increase in T_{reg} cells. To ensure that EAE resistance was not caused by a non-specific T cell defect, Batf$^{+/+}$ naïve CD4$^+$ T cells were adoptively transferred into Batf$^{-/-}$ mice prior to MOG administration.[4] Under these conditions, Batf$^{-/-}$ mice were no longer resistant to EAE, further supporting the involvement of Batf-dependent signaling in EAE development.

Using retroviral expression, Schraml *et al.* unveiled a synergistic relationship between RORγt and Batf in the production of IL-17.[4] Further studies showed that although early induction of RORγt and RORα was normal in Batf$^{-/-}$ T cells, Batf was required to maintain the prolonged increase in RORγt and RORα expression observed in wild-type cells. The authors also demonstrated that Batf preferentially forms a heterodimer with JunB in Th17 cells, and this complex binds to the promoter regions of IL-17, IL-21, and IL-22 to positively regulate expression. Collectively, data from these experiments suggest that Batf is an essential factor for the differentiation of Th17 cells and the production of IL-17.

Successful therapies for autoimmune disease must specifically inhibit pathological inflammation without inducing generalized immunosuppression. The most commonly prescribed treatment for MS is IFN-β, although this approach is only moderately effective, attenuating disease conditions by approximately 35%.[6] Using the EAE mouse model, two recent studies demonstrated that the underlying mechanism of IFN-β action is dependent on antigen-presenting cells secreting IL-27.[7,8] IL-27 promotes the production of IL-10 which expands T_{reg} populations and inhibits Th17 differentiation. Consistent with these observations, a dichotomy in the generation of T_{reg} and Th17 cells has been previously reported.[9] Further supporting Th17 cells as a direct pharmacological target, treatment with the small molecule halofuginone selectively inhibited Th17 cell differentiation and prevented EAE.[10] Encouragingly, positive findings from EAE studies may also translate to other autoimmune diseases. For example, a selective EP4 prostaglandin receptor antagonist inhibited Th17 cell expansion and suppressed disease progression in models of both EAE and contact hypersensitivity (CHS).[11]

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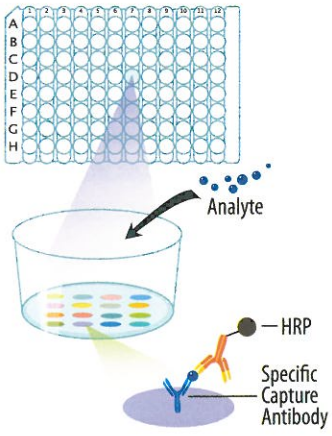
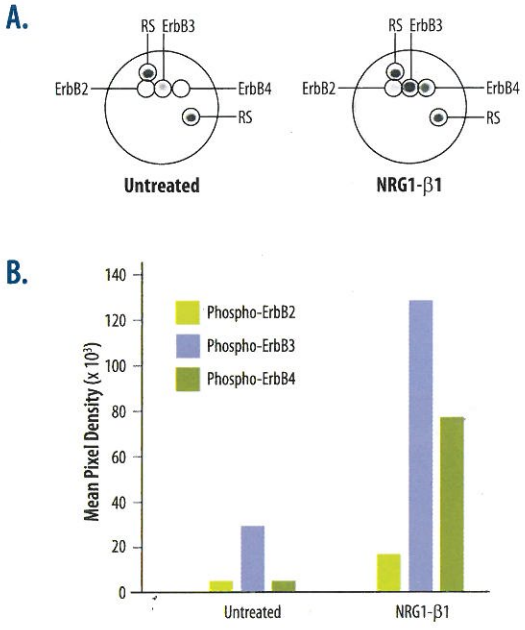


*Compatible imaging systems include Quansys Biosciences Q-View™ Imager; Alpha Innotech FluorChem® HD2 and FC2; BioRad® Versa Doc™ 4000, and ChemiDoc™ XRS; Fujifilm LAS-3000, LAS-3000 Mini, and Aushon BioSystems SearchLight® imager. Free acquisition software is available from Quansys Biosciences.



NRG1-β1 Induces Phosphorylation of ErbB Family Receptors in Breast Cancer Cells. A. Cell lysates prepared from MDA-MB-453 human breast cancer cells, untreated or treated with recombinant human NRG1-β1 (Catalog # 396-HB) for 5 minutes, were examined for the phosphorylation of 16 different receptor tyrosine kinases using the Proteome Profiler *96* Human Phospho-RTK Antibody Array 1 (Catalog # ARZ001). Changes in phosphorylation are highlighted in images taken from two microplate wells. RS=Reference Spot. **B.** Histogram profiles for receptor tyrosine kinases exhibiting significant phosphorylation were generated by quantifying the mean spot pixel densities from individual antibody spots using analytical software.

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