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INSIDE

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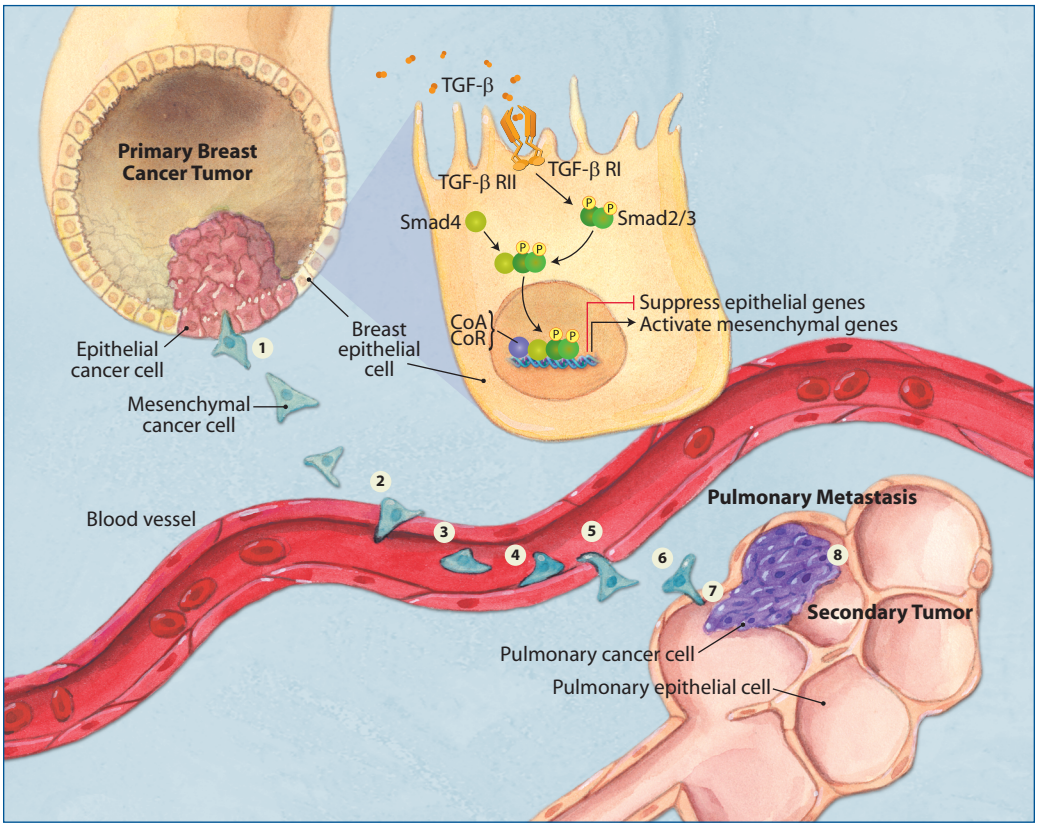


Breast cancer metastasis is dependent on EMT. Epithelial cancer cells in a breast milk duct undergo epithelial-to-mesenchymal transition (EMT) to acquire a more migratory mesenchymal phenotype. EMT induced by TGF-β suppresses epithelial genes and promotes the expression of mesenchymal proteins. Highly motile mesenchymal-like breast cancer cells invade pulmonary epithelia and proliferate as secondary tumors. This process requires EMT (1), mesenchymal cell intravasation (2), migration through the vasculature (3), adherence (4), extravasation (5), invasion of a secondary tissue (6), mesenchymal-to-epithelial transition (7), and distal proliferation (8).

TGF-β-Induced Epithelial-to-Mesenchymal Transition Promotes Breast Cancer Progression

In the U.S. alone, approximately 250,000 women are diagnosed with breast cancer every year.[1] Forty thousand women will die of the disease, which means that only lung carcinomas cause more cancer-related deaths. Critically, the response to therapy and clinical course are dependent on the subtype of tumor diagnosed.[2] For example, luminal A and luminal B type breast cancers are characterized by a low chance of metastasis and relatively good clinical outcomes. However, basal-like breast cancers (BLBC) are highly invasive, progress aggressively to distal tumors, and are associated with poor prognoses.

The invasive nature of each tumor subtype is dependent on epithelial cells increasing their capacity for migration through a process known as the epithelial-to-mesenchymal transition (EMT).[3] During EMT, epithelial cancer cells shed their epithelial characteristics and acquire more migratory mesenchymal cell-like properties. The reverse process, mesenchymal-to-epithelial transition (MET), facilitates the subsequent integration of cells at secondary locations. Recent studies have focused on the role of TGF-β, an established inducer of EMT, in breast cancer progression.[4]

Many groups have shown that TGF-β can induce EMT, but the precise signaling cascades involved are not completely understood.[5-7] Classic TGF-β signaling requires binding of TGF-β to type II TGF-β receptors, transphosphorylation of type I receptors, and subsequent phosphorylation of Smad2 and Smad3. Phosphorylated Smad2/3 forms a trimer with Smad4 that then translocates to the nucleus and interacts with transcription factors, co-activators, and co-repressors to suppress epithelial genes and promote the

Continued on page 7

The Nlrp3 Inflammasome & IL-18 Regulate Intestinal Homeostasis

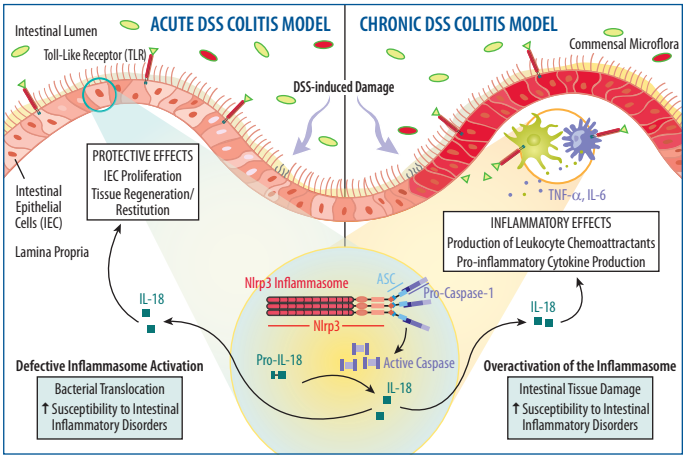
Nod-like receptors (NLRs) are intracellular pattern recognition receptors that are responsible for detecting invading pathogens and activating the innate immune response. Upon recognition of microbial components, some NLRs form cytoplasmic, multiprotein complexes known as inflammasomes that serve as platforms for the recruitment, cleavage, and activation of inflammatory caspases. Inflammasome activation of Caspase-1 is essential for the maturation and secretion of IL-1β and IL-18, two closely related IL-1 family cytokines that function as key mediators of the host immune response.

Several reports have established a link between defects in the inflammasome pathway and pathological conditions. The autoinflammatory disorders, Muckle-Wells Syndrome (MWS), Familial Cold Autoinflammatory Syndrome (FACS), and Neonatal-Onset Multisystem Inflammatory Disease (NOMID), are associated with mutations in *NLRP3* that lead to constitutive activation of the NLRP3 inflammasome. In contrast, single nucleotide polymorphisms that reduce *NLRP3* expression have recently been shown to be associated with an increased susceptibility to inflammatory bowel disease (IBD).[1] While autoinflammatory disorders seem to be primarily caused by elevated levels of active IL-1β, IL-18 is thought to be the critical effector molecule in intestinal disorders. This is based on the paradoxical findings that both a lack of IL-18 and IL-18 overexpression in experimental mouse models are associated with exacerbated intestinal inflammation in response to induced colonic epithelial damage.[2-5] In addition, IL-18 in humans is upregulated in colon tissue from patients with Crohn's disease.[6] Together, these observations imply that IL-18 may have both protective and detrimental effects in regulating mucosal immunity.

Two recent studies in mice provide further evidence that the Nlrp3 inflammasome, Caspase-1, and IL-18 are required for protection against colitis.[7,8] This was revealed by investigating the phenotypes of mice lacking different components of the Nlrp3 inflammasome following a five day treatment with dextran sodium sulfate (DSS), a polysaccharide that is toxic to the colonic epithelium.[7,8] Treatment of *Caspase-1*$^{-/-}$ or *Nlrp3*$^{-/-}$ mice with DSS led to intestinal bleeding, shortening of the colon, a significant increase in weight loss and lower survival rates compared to DSS-treated wild-type mice. Following DSS treatment, *Caspase-1*$^{-/-}$ and *Nlrp3*$^{-/-}$ mice also showed signs of increased bacterial invasion in the mesenteric lymph nodes, colon, and spleen or liver. The high levels of bacterial dissemination suggest that the inflammasome pathway is required for re-establishing the integrity of the epithelial layer following intestinal tissue damage.[8]

To determine whether intestinal tissue repair was dependent on IL-1β or IL-18, the concentration of each cytokine in the serum of DSS-treated wild-type mice was examined.[7,8] While the levels of IL-1β increased modestly in response to DSS, IL-18 levels increased dramatically, both in the serum and in the colon itself. IL-18 was produced primarily by nonhematopoietic cells, rather than macrophages or dendritic cells present in the lamina propria.[7] To determine the functional significance of increased IL-18 production, recombinant IL-18 was injected into *Caspase-1*$^{-/-}$ mice, zero to two days following DSS treatment.[7] The addition of exogenous IL-18 allowed DSS-treated, Caspase-1-deficient mice to survive and maintain a stable body weight, demonstrating that IL-18 is the key factor required for protection against DSS-induced colitis. These results suggest that reduced IL-18 production by intestinal epithelial cells following acute activation may compromise the mucosal barrier and increase susceptibility to intestinal inflammatory disorders.

Chronic inflammation associated with IBD increases the risk of developing colorectal cancer.[9] Significantly, a connection between the inflammasome pathway and colitis-associated colorectal cancer has also recently been identified.[7,10] Consistent with the inflammasome playing a protective role, Allen *et al.* demonstrated that mice lacking Caspase-1, ASC, or Nlrp3 were more susceptible than wild-type mice to inflammation-associated colorectal tumor development induced by the procarcinogen azoxymethane and recurring cycles of DSS administration.[10] In contrast, mice lacking Caspase-12, a negative regulator of Caspase-1, also displayed enhanced tumorigenesis following a similar treatment regimen.[7] While together these studies clearly establish a connection between the inflammasome and colitis-associated tumorigenesis, further research is necessary to clarify the regulatory mechanisms by which the inflammasome pathway can both inhibit and promote intestinal inflammation. Understanding these mechanisms may have therapeutic implications for IBD and colitis-associated colorectal cancer.



Nlrp3-mediated IL-18 Secretion Can Have Protective or Destructive Effects in the Intestine. Current data is consistent with a model that suggests that the Nlrp3 inflammasome can have either protective or deleterious effects in the intestine depending on the cell type in which it is activated. In response to acute colitis induced by dextran sodium sulfate (DSS), inflammasome activation in intestinal epithelial cells (IECs) and the subsequent secretion of IL-18 are necessary for IEC proliferation and tissue regeneration. Inflammasome activity in IECs limits mucosal damage and prevents stimulation of immune cells in the lamina propria. In contrast, chronic DSS exposure results in inflammasome activation in intestinal immune cells, potentially leading to the overproduction of inflammatory cytokines and subsequent tissue destruction.

References

1. Villani, A-C. *et al.* (2009) Nat. Genet. **41**:71.
2. Ishikura, T. *et al.* (2003) J. Gastroenterol. Hepatol. **18**:960.
3. Sivakumar, P.V. *et al.* (2002) Gut **50**:812.
4. Takagi, H. *et al.* (2003) Scand. J. Gasteroenterol. **38**:837.
5. Coruh, B. *et al.* (2001) Gasteroenterology **120**:A-122.
6. Pizarro, T.T. *et al.* (1999) J. Immunol. **162**:6829.
7. Dupaul-Chicoine, J. (2010) Immunity **32**:367.
8. Zaki, M. H. *et al.* (2010) Immunity **32**:379.
9. Clevers, H. (2004) Cell **118**:671.
10. Allen, I.C. *et al.* (2010) J. Exp. Med. **207**:1045.

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Adiponectin Signaling: The Calcium Connection

Adiponectin, also known as Acrp30, is an adipocytokine that positively regulates glucose and lipid metabolism by increasing insulin sensitivity, stimulating fatty acid oxidation and glucose uptake, and suppressing hepatic glucose production. Adiponectin deficiency is often associated with obesity and is highly correlated with insulin resistance, mitochondrial dysfunction, and dyslipidemia.[1,2] Over the past 15 years, a significant amount of research has been dedicated to determining the mechanisms by which Adiponectin functions. In 2003, Yamauchi *et al.* identified two Adiponectin receptors, Adipo R1 and Adipo R2.[3] While subsequent data from this group and others have clearly shown that these receptors bind Adiponectin and mediate its metabolic effects, details of how they do so have remained unclear.[2-5]

A recently published paper from the group that originally cloned Adipo R1 and R2 now takes a significant step toward elucidating the Adipo R1 signaling pathway.[6] This was accomplished using mice in which Adipo R1 was specifically deleted in skeletal muscle (muscle-Adipo R1KO). Adipo R1 is the primary Adiponectin receptor expressed in skeletal muscle, the major glucose-utilizing tissue in the body.[2-4] *In vivo* data from muscle-Adipo R1KO mice confirm the involvement of Adipo R1 in glucose tolerance and insulin sensitivity. This was demonstrated both by the presence of notably higher plasma glucose and insulin levels in fed muscle-Adipo R1KO mice compared to wild-type mice, and by significant changes in insulin-stimulated phosphorylation of signaling molecules such as IRS-1, Akt, p70 S6 kinase, and JNK.[6] Muscle-Adipo R1KO mice also displayed diminished mitochondrial biogenesis, as shown by decreased activity of the transcription factor PGC1α and reduced quantities of mitochondria-specific proteins and DNA.[2,6] As often accompanies insulin resistance, fatty acid oxidation was impaired, and oxidative stress was increased in the absence of skeletal muscle Adipo R1. Interestingly, exercise was able to improve these metabolic pathways, even in the absence of muscle Adipo R1, indicating that exercise can partially compensate for inadequate Adiponectin signaling.[6]

To determine the signaling defects responsible for the phenotypic changes in muscle-Adipo R1KO mice, thorough *in vitro* studies were performed using small interfering RNAs (siRNAs), and specific inhibitors to knock down the expression, or function, of metabolic regulatory molecules, such as Adipo R1 and R2, AMPKα1 and AMPKα2, CAMKKβ, PGC1α, SIRT1, and LKB1 in normal mouse myocytes.[6] Inhibition of Adipo R1, CAMKKβ, PGC1α, or both AMPKα1 and AMPKα2 reduced Adiponectin-induced mitochondrial biogenesis. In addition, Adipo R1 and CAMKKβ siRNA suppressed the increase in PGC1α expression that was induced by Adiponectin in normal mouse myocytes. Notably, both the suppression of Adipo R1 expression by siRNA, and the deletion of Adipo R1 in muscle-Adipo R1KO mice led to a defect in the influx of extracellular calcium that was observed in normal myocytes following Adiponectin treatment. This Adiponectin-mediated calcium influx is required for CaMKKβ activation, which in turn affects PGC1α through multiple pathways. First, activated CaMKKβ increases the expression of PGC1α, and second, it phosphorylates and activates AMPK. Activated AMPK increases the cellular NAD^+/NADH ratio, leading to activation of the SIRT1 deacetylase.[6,7] Both phosphorylation of PGC1α by AMPK and its deacetylation by SIRT1 enhance the transcriptional activity of PGC1α, which is essential for the expression of proteins involved in mitochondrial biogenesis.[6,8]

While these results shed new light on the cellular events associated with Adiponectin/Adipo R1 signaling, how Adipo R1 induces an influx of extracellular calcium still needs to be resolved. Four molecules are known to bind the Adipo R1 intracellular domain, including the adaptor proteins RACK1 and APPL1, the kinase regulatory subunit CK2β, and the endoplasmic reticulum protein ERp46. Although RACK1, CK2β, and ERp46 are all proposed to modulate Adiponectin signaling, their influence on calcium influx is unknown.[9-11] APPL1 is known to enhance signaling by Akt, a kinase required for the insulin signaling pathway, but this effect is thought to be independent of CaMKK.[5] TRPC3, the skeletal muscle T-tubule cation channel has been shown to modulate insulin-mediated glucose uptake, but connections with Adipo R1 signals are unknown.[12] Identifying the critical intracellular Adiponectin binding proteins that connect Adipo R1 to the calcium influx is one of the final steps required for a clear understanding of the Adiponectin signaling pathway in skeletal muscle.

References

1. Felder, T.K. *et al.* (2010) Int. J. Obes. (Lond) **34**:846.
2. Civitarese, A.E. *et al.* (2006) Cell Metab. **4**:75.
3. Yamauchi, T. *et al.* (2003) Nature **423**:762.
4. Yamauchi, T. *et al.* (2007) Nat. Med. **13**:332.
5. Zhou, L. *et al.* (2009) J. Biol. Chem. **284**:22426.
6. Iwabu, M. *et al.* (2010) Nature **464**:1313.
7. Canto, C. *et al.* (2010) Cell Metab. **11**:213.
8. Aquilano, K. *et al.* (2010) J. Biol. Chem. **285**:21590.
9. Xu, Y. *et al.* (2009) Biochem. Biophys. Res. Commun. **378**:95.
10. Charlton, H.K. *et al.* (2010) Biochem. Biophys. Res. Commun. **392**:234.
11. Heiker, J.T. *et al.* (2009) Cell. Signal. **21**:936.
12. Lanner, J.T. *et al.* (2009) FASEB J. **23**:1728.

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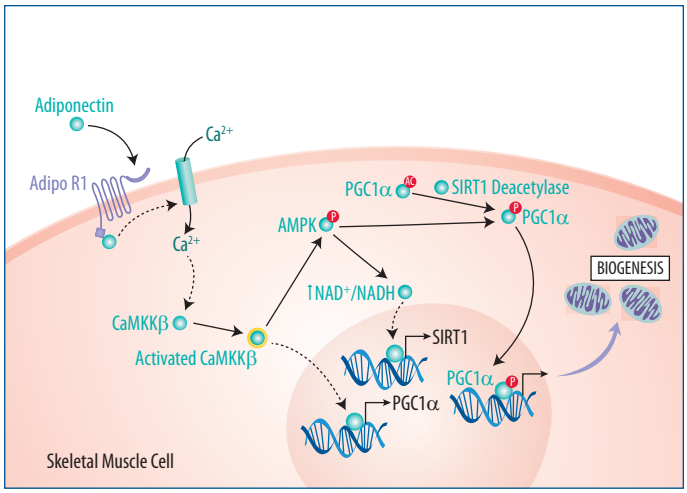

Adiponectin Signaling Promotes the Activation of PGC1α and Mitochondrial Biogenesis. Adiponectin binding to Adipo R1 causes a calcium influx in skeletal muscle that activates CaMKKβ. Activated CaMKKβ induces the expression and activation of PGC1α via a cascade involving the activation of AMPK and increased production of the SIRT1 deacetylase. AMPK phosphorylation and SIRT1 deacetylation of PGC1α increase its transcriptional activity, leading to increased mitochondrial biogenesis.

CCL21 & Tumor Immune Evasion

Tumor development and growth depends on the ability of tumor cells to evade the host's immune system. Tumor cells employ strategies to impede anti-tumor immune responses, including secretion of immunosuppressive factors and activation of negative regulatory pathways.[1] Recently, Shields *et al.* reported that invasive tumor cells secrete CCL21.[2] This group now describes a novel mechanism by which CCL21-secreting tumors transform their outer layer into lymphoid-like tissue to create a tolerant stromal microenvironment that promotes immune system evasion.[3]

Shields *et al.* generated mouse melanoma cell sublines expressing different amounts of CCL21 using lentiviral expression and small hairpin RNA (shRNA).[3] Murine-derived B16-F10 melanoma cells were transduced with lentivirus carrying a secondary lymphoid tissue-expressed form of murine *CCL21* cDNA (*CCL21-Ser/CCL21a*),[4,5] murine CCL21 shRNA, or a scrambled shRNA as a control. CCL21 secretion by these cells was either up-regulated ($CCL21^{high}$) or down-regulated ($CCL21^{low}$), respectively, or remained unchanged (control). The melanoma cell sublines were transplanted into immune competent syngeneic mice. Upon examination of the resulting tumors, the authors found that CCL21-secreting tumors (control and $CCL21^{high}$) grew significantly larger than $CCL21^{low}$ tumors. Further investigation revealed that the microenvironments of CCL21-secreting tumors had been reorganized to resemble lymphoid-like tissue.[3]

Lymphoid tissues are composed of a highly organized network of stromal cells that bring foreign antigens and immune cells into close contact to initiate adaptive immune responses. The formation of lymphoid tissues is coordinated by lymphoid tissue-induced (LTi) cells, which promote the localized expression of chemokines. LTi cells stimulate lymphoid stromal cells, such as fibroblast-like reticular cells (FRCs), to release chemokines that recruit antigen-presenting dendritic cells and lymphocytes into the lymphoid tissue.[6] FRCs secrete CCL19 and CCL21, stimulating the recruitment of $CCR7^+$ cells, such as naïve and memory T cells, mature dendritic cells, and LTi cells.[6-10] The microenvironments of CCL21-secreting tumors displayed characteristics of lymph tissue. LTi cells were present in the microenvironments of both control and $CCL21^{high}$ tumors, but were not associated with tumors expressing low levels of CCL21.[3] In addition, the lymphoid stroma-associated markers, gp38 and ER-TR7, and the high endothelial venule marker, peripheral node addressin (PNAd), were expressed in the stroma surrounding $CCL21^+$ tumors, but were absent from the periphery of $CCL21^{low}$ tumors.[3]

Though it seems counterproductive for tumors to create a peripheral environment that is similar to immune tissue, lymphoid tissue can promote immune tolerance. For example, lymph node stromal cells present peripheral tissue antigens to circulating T cells to induce peripheral tolerance.[11] Thus, it is plausible that the newly created lymphoid-like stromal structures aid tumor cells in immune system evasion and consequently, enhance tumor growth. Shields *et al.* uncovered several potential mechanisms that CCL21-secreting tumor cells may utilize to induce immune tolerance.[3] CCL21-secreting tumors recruited more $CD11b^+CD11c^-F4/80^-Gr1^{high}$ myeloid-derived suppressor cells (MDSCs) and regulatory T (Treg) cells. Additionally, two factors that facilitate tumor growth, indoleamine 2,3-dioxygenase (IDO) and complement receptor 1-related gene/protein y (Crry), were expressed by CCL21-secreting tumors, but were not present in $CCL21^{low}$ tumors.[3] $CCL21^{low}$ tumors also recruited more mature and cytotoxic T cells specific for melanoma antigen, and had elevated levels of IFN-γ, IL-2, and IL-4, cytokines associated with anti-tumor immunity and cytotoxic T cell responses. This research gives new insight into how tumor cells influence the immune system by inducing changes in their microenvironment, and provides new avenues for possible cancer treatments, including inhibition of CCL21 secretion by tumor cells or suppression of LTi cell function.

References

1. Rabinovich, G.A. *et al.* (2007) Ann. Rev. Immunol. **25**:267.
2. Shields, J.D. *et al.* (2007) Cancer Cell **11**:526.
3. Shields, J.D. *et al.* (2010) Science **328**:749.
4. Vassileva, G. *et al.* (1999) J. Exp. Med. **190**:1183.
5. Nakano, H. & M.D. Gunn (2001) J. Immunol. **166**:361.
6. Drayton, D.L. *et al.* (2006) Nat. Immunol. **7**:344.
7. Luther, S.A. *et al.* (2002) J. Immunol. **169**:424.
8. Weninger, W. *et al.* (2003) J. Immunol. **170**:4638.
9. van de Pavert, S.A. *et al.* (2009) Nat. Immunol. **10**:1193.
10. Mueller, S.N. & R.N. Germain (2009) Nat. Rev. Immunol. **9**:618.
11. Lee, J.W. *et al.* (2007) Nat. Immunol. **8**:181.
12. Liu, V.C. *et al.* (2007) J. Immunol. **178**:2883.
13. Prendergast, G.C. (2008) Oncogene **27**:3889.
14. Yoshida, N. *et al.* (2008) Clin. Cancer Res. **14**:7251.
15. Caragine, T.A. *et al.* (2002) Cancer Res. **62**:1110.
16. Fukumura, D. *et al.* (2006) Nat. Rev. Cancer **6**:521.

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Cells in the Tumor Microenvironment Promote Immune System Evasion

Cell Type	Proposed Role in Tumor Immune Tolerance
LTi Cell	• Initiates reorganization of the tumor microenvironment
FRC	• Forms the lymphoid-like stroma surrounding the tumor • Expresses Crry
MDSC	• Expresses high levels of iNOS
nTreg/iTreg Cell	• Inhibits immune cells • Secretes TGF-β1
Dendritic Cell	• Expresses IDO
Tumor Cell	• Expresses IDO • Expresses Crry • Secretes TGF-β1

Cells Present in the Microenvironments of CCL21-Secreting Tumors Promote Immune Tolerance. Tumor cells secrete CCL21 and recruit $CCR7^+$ cells that restructure the tumor microenvironment to resemble the stroma of lymph tissue. The cells present in the new microenvironment use a variety of mechanisms to promote immune tolerance and facilitate tumor growth. Naturally-derived Treg (nTreg) and tumor cells secrete TGF-β1, which suppresses T cell function and converts naïve T cells into induced Treg (iTreg) cells.[12] Tumor and dendritic cells express IDO, the rate-limiting enzyme in tryptophan catabolism. IDO suppresses T cell functions by depleting tryptophan stores and increasing local concentrations of the pro-apoptotic, tryptophan breakdown product, N-formylkynurenine.[13] Tumor and fibroblast-like reticular cells (FRCs) express Crry, a membrane-associated complement regulatory protein that protects tumor cells from the complement system by inhibiting C3/C5 convertase activity. Both IDO and Crry facilitate tumor growth.[14,15] Myeloid-derived suppressor cells (MDSCs) express inducible nitric oxide synthase (iNOS), which produces high concentrations of nitric oxide (NO). NO reduces lymphocyte reactions and promotes tumor cell proliferation by inducing the expression of angiogenic factors.[16]

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

1. Weiss, J *et al.* (2009) Successful immunotherapy with IL-2/anti-CD40 induces the chemokine-mediated mitigation of an immunosuppressive tumor microenvironment. Proc. Natl. Acad. Sci. USA **106**:19455.

   Mouse CCL17/TARC Quantikine® ELISA Kit (Catalog # MCC170)

   Mouse CCL22/MDC Quantikine ELISA Kit (Catalog # MCC220)

   Sample: Mouse tumor homogenate
   Application: ELISA

2. Horst, D. *et al.* (2009) Invasion associated up-regulation of nuclear factor κB target genes in colorectal cancer. Cancer **115**:4946.

   Human CXCL1/GROα Monoclonal Antibody (Catalog # MAB275)

   Human CCL5/RANTES Antigen Affinity-purified Polyclonal Antibody (Catalog # AF-278-NA)

   Sample: Human colorectal cancer
   Application: Immunohistochemistry

3. Fujimoto, H. *et al.* (2009) Stromal MCP-1 in mammary tumors induces tumor-associated macrophage infiltration and contributes to tumor progression. Int. J. Cancer **125**:1276.

   Human CCL2/MCP-1 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF-279-NA)

   Sample: Human breast cancer tumor grown in mouse
   Application: Immunohistochemistry

   Mouse Gr-1 (Ly-6G) Monoclonal Antibody (Catalog # MAB1037)

   Sample: Mouse infiltrating macrophages in human breast cancer tumor
   Application: Immunohistochemistry

   Mouse CCL2/JE/MCP-1 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF-479-NA)

   Sample: Mouse infiltrating macrophages in human breast cancer tumor
   Application: Immunohistochemistry

   Sample: Mouse macrophages
   Application: Neutralization

   Sample: Mouse
   Application: *In vivo*

   Recombinant Mouse CCL2/JE/MCP-1 (Catalog # 479-JE)

   Sample: Mouse macrophages
   Application: Chemotaxis

4. Singh, S. *et al.* (2009) Serum CXCL13 positively correlates with prostatic disease, prostate-specific antigen and mediates prostate cancer cell invasion, integrin clustering and cell adhesion. Cancer Lett. **283**:29.

   Human CXCR5 (BLR-1) Monoclonal Antibody (Catalog # MAB190)

   Sample: PCa human prostate cancer cell line
   Application: Neutralization

   Human CXCL13/BLC/BCA-1 Quantikine ELISA Kit (Catalog # DCX130)

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   Human IL-6 Quantikine High Sensitivity ELISA Kit (Catalog # HS600B)

   Human TNF-α Quantikine High Sensitivity ELISA (Catalog # HSTA00D)

   Sample: Human serum
   Application: ELISA

5. Busuttil, A. *et al.* (2009) CXCR3 ligands are augmented during the pathogenesis of pulmonary sarcoidosis. Eur. Respir. J. **34**:676.

   Human CXCR3 Monoclonal Antibody (Catalog # MAB160)

   Human CXCL9/MIG Monoclonal Antibody (Catalog # MAB392)

   Sample: Human lung
   Application: Immunohistochemistry

   Human CXCL9/MIG Quantikine ELISA Kit (Catalog # DCX900)

   Human CXCL10/IP-10 Quantikine ELISA Kit (Catalog # DIP100)

   Human CXCL11/I-TAC Quantikine ELISA Kit (Catalog # DCX110)

   Sample: Human bronchoalveolar lavage fluid
   Application: ELISA

6. Lu, X. *et al.* (2009) Chemokine (C-C Motif) ligand 2 engages $CCR2^+$ stromal cells of monocytic origin to promote breast cancer metastasis to lung and bone. J. Biol. Chem. **284**:29087.

   Human CCL2/MCP-1 Monoclonal Antibody (Catalog # MAB279)

   Sample: Concentrated conditioned media from H29 human lung cancer cell line
   Application: Western blot

   Human CCL2/MCP-1 Monoclonal Antibody (Catalog # MAB679)

   Sample: Human tumor grown in mouse
   Application: *In vivo*

7. Jaafar, F. *et al.* (2009) Correlation of CXCL12 expression and $FoxP3^+$ cell infiltration with human papillomavirus infection and clinicopathological progression of cervical cancer. Am. J. Pathol. **175**:1525.

   Human CXCL12/SDF-1α Quantikine ELISA Kit (Catalog # DSA00)

   Sample: Human homogenized cervical tissue
   Application: ELISA

   Human/Mouse CXCL12/SDF-1 Monoclonal Antibody (Catalog # MAB350)

   Sample: Human cervix
   Application: Immunohistochemistry and Western blot

8. Wallace, A. *et al.* (2009) Prostaglandin F2α-F prostanoid receptor signaling promotes neutrophil chemotaxis via chemokine (C-X-C motif) ligand 1 in endometrial adenocarcinoma. Cancer Res. **69**:5726.

   Human CXCR2/IL-8 RB Monoclonal Antibody (Catalog # MAB331)

   Mouse Gr-1 (Ly-6G) Monoclonal Antibody (Catalog # MAB1037)

   Sample: Human endometrial tissue
   Application: Immunohistochemistry

   Human CXCL1/GROα Quantikine ELISA Kit (Catalog # DGR00)

   Sample: Ishikawa human endometrial adenocarcinoma cell culture supernates
   Application: ELISA

9. Franciszkiewicz, K. *et al.* (2009) Intratumoral induction of CD103 triggers tumor-specific CTL function and CCR5-dependent T cell retention. Cancer Res. **69**:6249.

   Human CCR5 Monoclonal Antibody (Catalog # MAB182)

   Human CCR6 Monoclonal Antibody (Catalog # MAB195)

   Human CXCR3 Monoclonal Antibody (Catalog # MAB160)

   Human CXCR4 (Fusin) Monoclonal Antibody (Catalog # MAB172)

   Human E-Cadherin Monoclonal Antibody (Catalog # MAB1838)

   Sample: Heu171 and H32-22 human non-small cell lung carcinoma cell lines
   Application: Flow cytometry

   Human CCL3/MIP-1α Monoclonal Antibody (Catalog # MAB270)

   Human CCL4/MIP-1β Monoclonal Antibody (Catalog # MAB271)

   Human CXCL9/MIG Monoclonal Antibody (Catalog # MAB392)

   Human CXCL10/IP-10 Monoclonal Antibody (Catalog # MAB266)

   Sample: Heu171 and H32-22 human non-small cell lung carcinoma cell lines
   Application: Immunohistochemistry

   Human CCL20/MIP-3α Quantikine ELISA Kit (Catalog # DM3A00)

   Human CXCL12/SDF-1α Quantikine ELISA Kit (Catalog # DSA00)

   Sample: Heu171 and H32-22 human non-small cell lung carcinoma cell culture supernates
   Application: ELISA

TECHNICAL NOTE:
R&D Systems MagCellect™ Human Blood Dendritic Cell Isolation Kit

Dendritic cells (DCs) provide a critical link between the innate and adaptive immune responses. These specialized cells have the ability to capture, process, and present antigens to naïve T cells to promote their differentiation and activation. While all DCs are capable of antigen presentation, DCs are not a homogeneous population of cells. Several DC subsets have been characterized that differ in their locations, phenotypes, and immunological functions. This plasticity allows them to differentially shape the immune response when presented with diverse microbial antigens or components of damaged cells.

Approximately 1-10% of peripheral blood mononuclear cells (PBMCs) in human blood are dendritic cells. This scarcity, along with a relatively low abundance of unique markers, and the continuous discovery of novel subpopulations, has made it a challenge to isolate DCs for functional studies. To address this challenge, R&D Systems has now developed a negative selection kit to enrich for DCs isolated from human blood. Unlike positive selection kits, the MagCellect Human Blood DC Isolation Kit (Catalog # MAGH110) leaves the isolated DC population untouched, reducing the risk of the cells being altered during the selection process. Flow cytometric analysis of the DC population isolated with the MagCellect Kit demonstrates that the cells are over 90% $CD14^-CD19^-BDCA1^+BDCA2^+BDCA3^+$ (Figure 1A). Of these, 2-8% are $BDCA2^+CD45^+$ plasmacytoid DCs (pDCs; Figure 1B), and 82-88% are myeloid DCs (mDCs). The mDC population is 20-40% $BDCA1^+CD11c^+$, 20-40% $BDCA3^+CD11c^+$, 10-20% $CD16^+CD11c^+$ and 10-25% $CD56^+MHCII^+$, indicating that the MagCellect Kit can be used to isolate the recently identified $CD56^+$ and $CD16^+$ mDC subpopulations (Figure 1C and 1D).

Functional analysis of the total enriched DC population confirms that the isolated cells maintain their ability to stimulate the proliferation of allogeneic T cells in a mixed leukocyte reaction assay (Figure 2). Since the isolated DCs remain untouched by antibodies throughout the enrichment procedure, they likely retain more functional capabilities than cells isolated using positive selection techniques. In addition, the MagCellect Kit relies on two antibody cocktails to tag unwanted cells and does not include a column, making it easier to use than other kits. R&D Systems new MagCellect Human Blood Myeloid Dendritic Cell Isolation Kit (Catalog # MAGH120) has similar benefits.

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

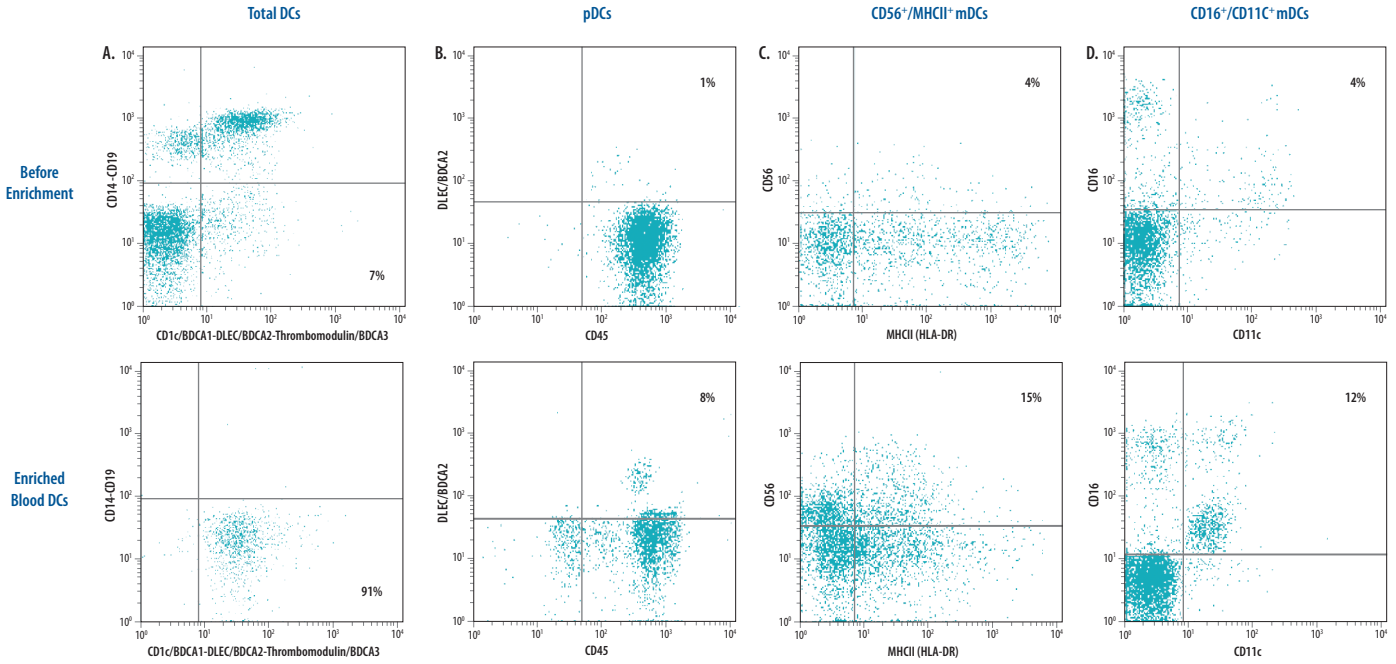

Figure 1. Analysis of Dendritic Cells Isolated using the MagCellect Human Blood Dendritic Cell Isolation Kit by Flow Cytometry. The MagCellect Human Blood Dendritic Cell Isolation Kit (Catalog # MAGH110) was used to enrich for total dendritic cells (DCs) obtained from human blood samples. Different DC subpopulations were detected, before (top) and after (bottom) enrichment, by flow cytometry using the indicated antibodies. **A.** Total DCs were detected using anti-CD14, anti-CD19, anti-CD1c/BDCA1, anti-DLEC/BDCA2, and anti-Thrombomodulin/BDCA3 antibodies. CD14 and CD19 are markers of monocytes and B cells, respectively. **B.** Plasmacytoid DCs (pDCs) were detected using anti-DLEC/BDCA2 and anti-CD45 antibodies. **C.** $CD56^+MHCII^+$ myeloid DCs (mDCs) were detected using anti-CD56 and anti-MHCII antibodies. **D.** $CD16^+CD11c^+$ mDCs were detected using anti-CD16 and anti-CD11c antibodies. Percentages of the subpopulations detected may vary due to donor variability.

TECHNICAL NOTE continued from page 6

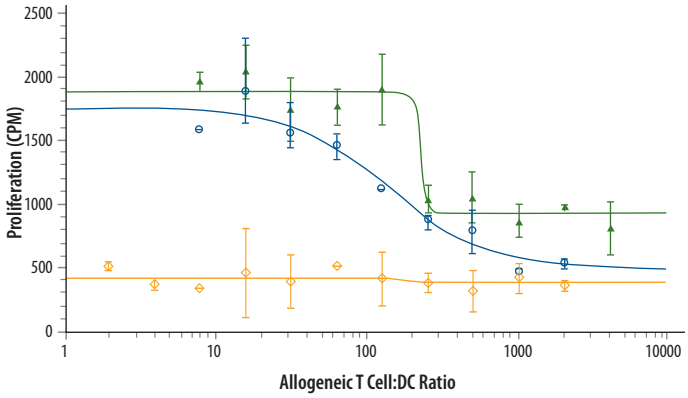

Figure 2. Dendritic Cells Isolated using the MagCellect Human Blood DC Isolation Kit Induce Allogeneic T Cell Proliferation. Dendritic cells were isolated from human blood using the MagCellect Human Blood Dendritic Cell Isolation Kit (Catalog # MAGH110). The ability of these cells to stimulate the proliferation of allogeneic T cells was assessed using the mixed leukocyte reaction assay. T cells were cultured either alone (gold line), or with serial dilutions of isolated DCs (blue line), or cultured monocyte-derived DCs as a positive control (green line), starting at a T cell:DC ratio of 8:1. T cell proliferation was measured after 3 days by ^{3}H-thymidine incorporation.

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

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Autumn Immunology Conference Booth # 109
Chicago, IL . . . . . November 19-22, 2010

San Antonio Breast Cancer Symposium Booth # 358
San Anton io, TX . . . . . December 8-12, 2010

American Society for Cell Biology
Philadelphia, PA . . . . . December 11-15, 2010

TGF-β-Induced EMT Promotes Breast Cancer Progression continued from page 1

expression of mesenchymal proteins. In addition, non-Smad signaling through activation of ERK MAP Kinases, Rho GTPases, and PI 3-Kinase/Akt has also been implicated in TGF-β-induced EMT.[8-10]

A recent paper investigated the hypothesis that Annexin A1 (AnxA1), an actin regulatory protein, is functionally involved in breast cancer progression.[11] de Graauw *et al.* observed consistently greater AnxA1 expression in BLBC-like, compared to luminal-like, breast cancer cell lines. Using AnxA1 small interfering RNA (siRNA), the authors could drive BLBC-like cells from a mesenchymal to an epithelial morphology, an effect that was reversed by ectopic AnxA1 expression. AnxA1 siRNA also reduced TGF-β-induced Smad2 phosphorylation and nuclear translocation of Smad4, indicating that AnxA1 was able to regulate TGF-β signaling. Further *in vitro* studies showed that expression of AnxA1 in the luminal-like MCF-7 human breast cancer cell line increased cell scattering and Smad3/4 transcriptional reporter activity, effects that could be blocked by the TGF-β receptor inhibitor SB-431542.

To extend their studies to an *in vivo* model, highly invasive 4T1 mouse breast cancer cells were injected into mouse mammary fat pads. In this model, knockdown of AnxA1 using short hairpin RNA (shRNA) had no effect on primary tumor growth, but significantly reduced the number of surface metastases in the lungs. To investigate the clinical relevance of these findings, de Graauw *et al.* examined tissue microarrays from breast cancer patients. Analysis of these samples revealed that AnxA1 expression correlated with pathological tumor grade, and was significantly higher in BLBC compared to other tumor subtypes.

Other groups have investigated Smad-dependent TGF-β signaling in the context of breast cancer progression. Recently, Araki *et al.* studied the effect of TGF-β on the p53 tumor suppressor protein.[12] The authors showed that TGF-β increased the expression of the E3 ubiquitin ligase human double minute 2 (HDM2) in a Smad3/4-dependent manner. HDM2 conjugates ubiquitin to p53, tagging it for degradation by the proteasome, and eliminating its capacity for tumor suppression. Using a mouse mammary epithelial cell line, Araki *et al.* reported similar changes in the expression of murine double minute 2 (MDM2) and p53 during TGF-β-induced EMT. Following examination of human clinical samples, the authors discovered a significant correlation between Smad3 activation and HDM2 levels in ductal and lobular breast carcinomas. Importantly, increased HDM2 expression and Smad3 activation were not detected in surrounding normal epithelial cells, supporting the specificity of these findings to breast cancer pathology.

Collectively, these recent studies reveal a paradoxical role for TGF-β in regard to breast cancer progression. Although TGF-β is known to suppress epithelial cell proliferation and therefore primary tumorigenesis, it is now believed to promote metastasis via the induction of EMT.[13] Elucidation of the signaling pathways involved may present a novel pharmacological target for the prevention of breast cancer progression via the inhibition of EMT.

References

1. American Cancer Society: www.cancer.org/Research/CancerFactsFigures
2. Sørlie, T. *et al.* (2001) Proc. Natl. Acad. Sci. USA **98**:10869.
3. Kalluri, R. & R.A. Weinberg (2009) J. Clin. Invest. **119**:1420.
4. Xu, J. *et al.* (2009) Cell Research **19**:156.
5. Sato, Y. *et al.* (2010) Am. J. Path. **771**:141.
6. Takahashi, E. *et al.* (2010) J. Biol. Chem. **285**:4060.
7. Vincent, T. *et al.* (2010) Nat. Cell. Biol. **11**:943.
8. Santibáñez, J.F. *et al.* (2010) FEBS Lett. **584**:2305.
9. Lee, J. *et al.* (2010) J. Biol. Chem. [Epub ahead of print]
10. Deng, B. *et al.* (2010) Mol. Cell. Biochem. **340**:21.
11. de Graauw, M. *et al.* (2010) Proc. Natl. Acad. Sci. USA **107**:6340.
12. Araki, S. *et al.* (2010) J. Clin. Invest. **120**:290.
13. Singh, G. *et al.* (2010) J. Biol. Chem. **285**:27241.

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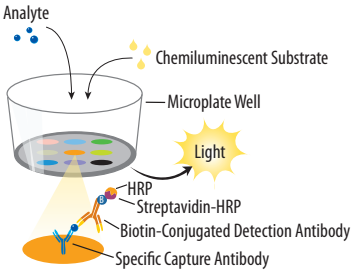
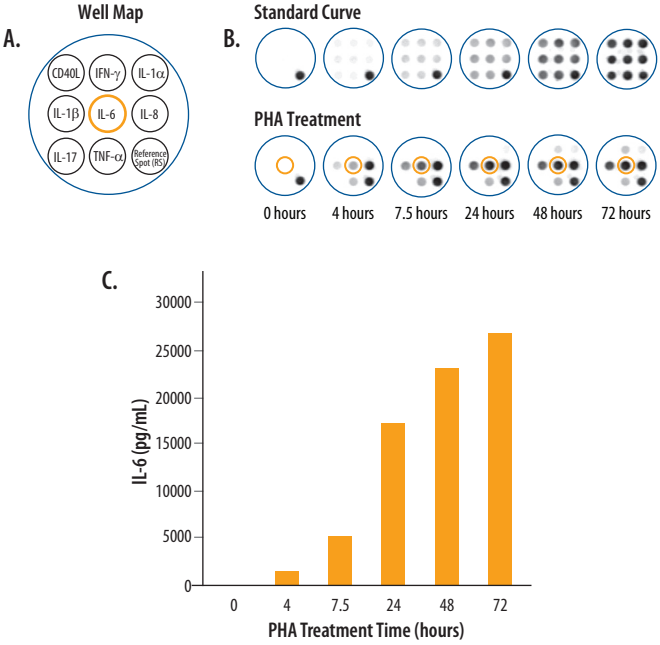
NEW TOOLS: Mosaic™ ELISA Human Cytokine Panel 1

R&D Systems has introduced a new format for quantitative multi-analyte profiling. The Mosaic ELISA Human Cytokine Panel 1 (Catalog # MEA001) is a 96-well microplate-based multiplex immunoassay that allows for the simultaneous quantification of 8 cytokines in a single sample of cell culture supernatant, serum, or plasma. The assay utilizes a microplate that has been pre-spotted in each well with antibodies that specifically recognize CD40L, IFN-γ,IL-1α, IL-1β, IL-6, IL-8, IL-17, and TNF-α. Samples are added to the wells and the target analytes are bound by their specific capture antibodies. Biotinylated detection antibodies, Streptavidin-HRP, and chemiluminescent substrate reagents are subsequently used to produce analyte-specific signals proportional to the amount of analyte bound to each individual capture antibody. Plates can be read using several common chemiluminescence camera systems.* By combining the specificity of the traditional two-site sandwich immunoassay with the high-throughput capabilities of multi-analyte profiling assays, the Mosaic ELISA provides an excellent alternative to performing multiple traditional ELISA experiments. For more information and instructional videos, please visit our website at **www.RnDSystems.com/go/Mosaic**.



Mosaic ELISA Human Cytokine Panel 1 Features:

✓ 96-well microplate format ✓ 8 analytes per well
✓ 320 data points in duplicate ✓ 25 µL sample size
✓ 4.5 hours/assay



Simultaneous Detection of Multiple Analytes using the Mosaic ELISA Human Cytokine Panel. Human peripheral blood mononuclear cells (PBMCs) were treated with PHA for the indicated times. Aliquots of the cell culture supernatants were removed and the Mosaic ELISA Human Cytokine Panel 1 (Catlaog # MEA001) was used to simultaneously quantify the levels of eight different cytokines. **A.** A well map indicating the spot locations of the analytes that can be detected using the Mosaic ELISA Human Cytokine Panel. The reference spot (RS) provides a strong positive signal for easy visualization of the well locations and spot alignment during data analysis. **B.** Representative images of individual wells for the standard curve and supernatants from PHA-treated PBMCs. PBMC supernatants were diluted 1:64 to ensure that the values for all of the analytes fell within the standard curve. **C.** A histogram profile for IL-6 was generated by analysis of the mean spot pixel density of the IL-6 spot in each well (circled in **B**).

* Compatible imaging systems tested by R&D 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.