

Literatures Introducing of Toll-like Receptor 4 (TLR4), Lipopolysaccharide (LPS)

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Abstract: Toll-like receptor 4 (TLR 4) is one of toll-like receptors that detects lipopolysaccharide (LPS) from Gram-negative bacteria. The various TLRs exhibit different expression patterns. TLR 4 is most abundantly expressed in placenta. Mutations of TLR 4 gene have been associated with differences in LPS responsiveness. The molecular weight of TLR 4 is approximately 95 kDa.

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Abbreviations: LPS, lipopolysaccharide; TLR, toll-like receptor

Introduction

Toll-like receptor 4 (TLR 4) is one of toll-like receptors that detects lipopolysaccharide (LPS) from Gram-negative bacteria and is important in the activation of the living bodies' innate immune system (Medzhitovm 1997; Rock, 1998). TLR 4 is a kind of cluster of differentiation 284 (CD284). The structure of TLRs is conserved from *Drosophila* to human beings and the structural and functional are similar in different species. They recognize pathogen-associated molecular patterns (PAMPs) that are expressed to respond infectious factor for the development of effective immunity. TLR 4 is expressed abundantly in the animal placenta. It cooperates with CD14 to regulate the signal transduction induced by LPS found in most gram-negative bacteria. The molecular weight of TLR 4 is approximately 95 kD. TLRs exist in both the vertebrates and invertebrates. TLRs are conserved components of the immune system. Toll-like receptors are one of the key molecules related to the immune system reacting to the microbial infections.

The name of Toll-like receptors is from their similarity to the protein coded by the Toll gene identified in *Drosophila* in 1985 by Christiane Nüsslein-Volhard. Toll is German for amazing or great. Mutants in the Toll gene were originally identified by 1995 Nobel Laureates Christiane Nüsslein-Volhard and Eric Wieschaus in the fruit fly *Drosophila melanogaster* in 1985, and cloned by the laboratory of Kathryn Anderson in 1988. Since then, 13 mammalian Toll genes have been identified. The Toll genes encode members of the Toll-like receptor class of proteins. In 1997, Charles Janeway and Ruslan Medzhitov showed that a Toll-like receptor could induce the activation of certain genes necessary for initiating an adaptive immune response. TLR 4 function as an LPS sensing receptor was discovered

by Bruce A. Beutler and colleagues. This identified TLR4 as one of the key components of the receptor for LPS.

In 1996, *Toll* was found by Jules A. Hoffmann and it was found having an essential role in the fly's immunity to fungal infection. The plant homologs were discovered by Pamela Ronald in 1995 and Thomas Boller in 2000. In flies, Toll was first identified as a gene important in embryogenesis to establish the dorsal-ventral axis. In 1996, Toll was found to have a role in the fly's immunity to fungal infections. Both mammalian and invertebrate require Toll genes for innate immunity.

Toll-like receptors in mammals were found in 1997 at Yale University by Ruslan Medzhitov and Charles Janeway (New Heaven, Connecticut, USA). Shizuo Akira and Bruce A. Beutler discovered that the Toll-like receptors (TLRs) act as the principal sensors of infection in mammals.

The first reported human Toll-like receptor was described by Nomura and colleagues in 1994, mapped to a chromosome by Taguchi and colleagues in 1996. Because the immune function of Toll in *Drosophila* was not then known, it was assumed that TIL might participate in mammalian development. In 1991, it was observed that a molecule with a clear role in immune function in mammals, the interleukin-1 (IL-1) receptor, also had homology to *drosophila* Toll; the cytoplasmic portions of both molecules were similar. On October 3, 2011, Dr. Beutler and Dr. Hoffmann were awarded the Nobel Prize in Medicine or Physiology for their TLR 4 work. Drs. Hoffmann and Akira received the Canada Gairdner International Award in 2011.

TLRs were identified also in the mammalian nervous system, which were detected on glia, neurons and on neural progenitor cells in which they regulate

cell-fate decision. Most mammalian species have 10 to 15 types of Toll-like receptors, and 13 TLRs (TLR1 to TLR13) have been identified in humans and mice together, and equivalent forms of many of these have been found in other mammalian species. TLR3 is the only Toll-like receptor which does not use the MyD88 dependent pathway. Its ligand is retroviral double-stranded RNA (dsRNA), which activates the TRIF dependent signalling pathway. TLR4 is the only TLR that uses all four adaptors. Complex consisting of TLR4, MD2 and LPS recruits TIR domain-containing adaptors TIRAP and MyD88 and thus initiates activation of NF κ B (early phase) and MAPK. TLR4-MD2-LPS complex then undergoes endocytosis and in endosome it forms a signalling complex with TRAM and TRIF adaptors. This TRIF-dependent pathway again leads to IRF3 activation and production of type I interferons, but it also activates late-phase NF κ B activation. Both late and early phase activation of NF κ B is required for production of inflammatory cytokines. TLR4 is involved in the development of contact allergy to nickel in humans. TLR ligands cause an IL-10-dependent inhibition of CD4 T-cell expansion and function by up-regulating PD-1 levels on monocytes, which leads to IL-10 production by monocytes after binding of PD-1 by PD-L.

TLRs seem to be involved only in the cytokine production and cellular activation in response to microbes, and do not play a significant role in the adhesion and phagocytosis of microorganisms. Toll-like receptors bind and become activated by different ligands, which, in turn, are located on different types of organisms or structures. They also have different adapters to respond to activation and are located sometimes at the cell surface and sometimes to internal cell compartments. Immune cells can produce signalling factors called cytokines, which trigger inflammation. In the case of a bacterial factor, the pathogen might be phagocytosed and digested, and its antigens presented to CD4⁺ T cells. In the case of a viral factor, the infected cell may shut off its protein synthesis and may undergo programmed cell death (apoptosis). Immune cells that have detected a virus may also release anti-viral factors such as interferons.

Mesenchymal stem cells (MSCs)-based regenerative therapy is regarded as an alternative method to treat the acute myocardial infarcted hearts. The efficiency of MSCs transplantation is limited by lower survival rate of engrafted MSCs. Yao et al found that 1.0 μ g/ml LPS could protect MSCs against apoptosis induced by oxidative stress and meanwhile enhance the proliferation of MSCs. Expression of vascular endothelial growth factor (VEGF) and phospho-Akt was increased in the infarcted myocardium after transplantation of LPS-preconditioned MSCs. LPS preconditioning enhanced

survival of engrafted MSCs, stimulated expression of VEGF and activated PI3K/Akt pathway. LPS preconditioning before MSCs transplantation resulted in superior therapeutic neovascularization and recovery of cardiac function. LPS preconditioning provided a novel strategy in maximizing biologic and functional properties of MSCs (Yao, Zhang et al. 2009).

Endotoxin LPS is a structural component of gram-negative bacteria membranes and a potent proinflammatory agent. Epidemiologic reports indicate that exposure to endotoxin can cause inflammatory airway diseases in agricultural workers and can exacerbate reactive airway disease in those with asthma and in wheezing children. A single exposure to aerosolized LPS can induce airflow obstruction that commences within min of challenge and persists for up to 48 h. Inhaled LPS leads to neutrophil recruitment and the release of proinflammatory molecules, including interleukin (IL), tumor necrosis factor (TNF), and the chemokines macrophage inflammatory protein-2, keratinocyte-derived chemokine (Hollingsworth, et al, 2004). Stimulation of unrestricted somatic stem cells with either lipopolysaccharide (LPS) or flagellin resulted in a marked increase of interleukin (IL)-6 and/or IL-8 production although levels differed significantly between both stimuli (van den Berk, Jansen et al. 2009). Apoptosis of implanted MSCs limits the efficiency of MSC therapy. Wang et al showed the ligands of TLRs could control the function of these cells. The appropriate treatments with LPS can protect MSCs from oxidative stress-induced apoptosis and improve the survival of MSCs via the TLR4 and PI3K/Akt pathway (Wang, Zhang et al. 2009).

The self-renewal and multilineage differentiation of embryonic stem cells (ESC) is largely governed by transcription factors or repressors. Extensive efforts have focused on elucidating critical factors that control the differentiation of specific cell lineages, for instance, myeloid lineages in hematopoietic development. Sharabi et al found that Twist-2, a basic helix-loop-helix (bHLH) transcription factor, plays a critical role in inhibiting the differentiation of ESC. Murine ES cells, in which Twist-2 expression is silenced by lentivirally delivered shRNA, exhibit an enhanced formation of primary embryoid bodies (EB) and enhanced differentiation into mesodermally derived hematopoietic colonies. Furthermore, Twist-2 silenced (LV-siTwist-2) ESC display significantly increased generation of myeloid lineages (Gr-1(+) and F4/80(+) cells) during in vitro hematopoietic differentiation. Treatment with the Toll-like receptor (TLR) 4 ligand synergistically stimulates the generation of primary EB formation as

well as of hematopoietic progenitors differentiated from LV-siTwist-2 ES cells (Sharabi, Lee et al. 2009).

Sepsis causes over 200,000 deaths yearly in the US; better treatments are urgently needed. Administering bone marrow stromal cells (BMSCs -- also known as mesenchymal stem cells) to mice before or shortly after inducing sepsis by cecal ligation and puncture reduced mortality and improved organ function. The beneficial effect of BMSCs was eliminated by macrophage depletion or pretreatment with antibodies specific for interleukin-10 (IL-10) or IL-10 receptor. Monocytes and/or macrophages from septic lungs made more IL-10 when prepared from mice treated with BMSCs versus untreated mice. Lipopolysaccharide (LPS)-stimulated macrophages produced more IL-10 when cultured with BMSCs, but this effect was eliminated if the BMSCs lacked the genes encoding Toll-like receptor 4, myeloid differentiation primary response gene-88, tumor necrosis factor (TNF) receptor-1a or cyclooxygenase-2. Our results suggest that BMSCs (activated by LPS or TNF-alpha) reprogram macrophages by releasing prostaglandin E(2) that acts on the macrophages through the prostaglandin EP2 and EP4 receptors. Because BMSCs have been successfully given to humans and can easily be cultured and might be used without human leukocyte antigen matching, we suggest that cultured, banked human BMSCs may be effective in treating sepsis in high-risk patient groups (Nemeth, Leelahavanichkul et al. 2009).

Activation of TLR4 by administration of LPS shortens the survival of skin allografts in mice treated with costimulation blockade through a CD8 T cell-dependent, MyD88-dependent, and type I IFN receptor-dependent pathway. The effect of TLR4 activation on the establishment of allogeneic hematopoietic chimerism in mice treated with costimulation blockade is not known. Using a costimulation blockade protocol based on a donor-specific transfusion (DST) and a short course of anti-CD154 mAb, we show that LPS administration at the time of DST matures host alloantigen-presenting dendritic cells, prevents the establishment of mixed allogeneic hematopoietic chimerism, and shortens survival of donor-specific skin allografts. LPS mediates its effects via a mechanism that involves both CD4(+) and CD8(+) T cells and results from signaling through either the MyD88 or the type I IFN receptor pathways. We also document that timing of LPS administration is critical, as injection of LPS 24 h before treatment with DST and anti-CD154 mAb does not prevent hematopoietic engraftment but administration the day after bone marrow transplantation does. We conclude that TLR4 activation prevents the induction of mixed allogeneic hematopoietic chimerism through type I IFN receptor

and MyD88-dependent signaling, which leads to the up-regulation of costimulatory molecules on host APCs and the generation of alloreactive T cells. These data suggest that distinct but overlapping cellular and molecular mechanisms control the ability of TLR agonists to block tolerance induction to hematopoietic and skin allografts in mice treated with costimulation blockade (Skrivuletz, Miller et al.).

Alcohol synergistically enhances the progression of liver disease and the risk for liver cancer caused by hepatitis C virus (HCV). However, the molecular mechanism of this synergy remains unclear. Here, we provide the first evidence that TLR4 is induced by hepatocyte-specific transgenic (Tg) expression of the HCV nonstructural protein NS5A, and this induction mediates synergistic liver damage and tumor formation by alcohol-induced endotoxemia. We also identify Nanog, the stem/progenitor cell marker, as a novel downstream gene up-regulated by TLR4 activation and the presence of CD133/Nanog-positive cells in liver tumors of alcohol-fed NS5A Tg mice. Transplantation of p53-deficient hepatic progenitor cells transduced with TLR4 results in liver tumor development in mice following repetitive LPS injection, but concomitant transduction of Nanog short-hairpin RNA abrogates this outcome. Taken together, our study demonstrates a TLR4-dependent mechanism of synergistic liver disease by HCV and alcohol and an obligatory role for Nanog, a TLR4 downstream gene, in HCV-induced liver oncogenesis enhanced by alcohol (Machida, Tsukamoto et al. 2009).

Caries-induced pulpitis is typically accompanied by an increase in dental pulp microvascular density. However, the mechanisms by which dental pulp cells recognize lipopolysaccharides (LPSs) remain unclear. We hypothesized that *Porphyromonas endodontalis* and *Escherichia coli* LPSs induce vascular endothelial growth factor (VEGF) expression in dental pulp stem cells (DPSC) and human dental pulp fibroblasts (HDPF) through mitogen-activated protein kinase (MAPK) signaling. ELISA, semi-quantitative RT-PCR, immunofluorescence, and Western blots were used. Here, we observed that LPSs induced VEGF expression in DPSC and HDPF cells, and both cell types express TLR-4. Notably, LPS-induced VEGF is associated with phosphorylation of protein kinase C (PKC zeta) and extracellular signal-regulator kinase (ERK1/2) and is dependent upon MAPK activation. Analysis of these data, collectively, unveils a signaling pathway responsible for synthesis of VEGF by pulp cells and suggests a novel therapeutic target for the management of vascular responses in teeth with pulpitis (Botero, Son et al).

The following are some related literatures references from Internet (Pubmed, etc):

Brudecki, L., D. A. Ferguson, et al. "Hematopoietic stem-progenitor cells restore immunoreactivity and improve survival in late sepsis." *Infect Immun* **80**(2): 602-11.

Sepsis progresses from an early/acute hyperinflammatory to a late/chronic hypoinflammatory phase with immunosuppression. As a result of this phenotypic switch, mortality in late sepsis from persistent primary infection or opportunistic new infection often exceeds that in acute sepsis. Emerging data support that persistence of the hypoinflammatory (hypo-responsive) effector immune cells during late sepsis might involve alterations in myeloid differentiation/maturation that generate circulating repressor macrophages that do not readily clear active infection. Here, we used a cecal ligation and puncture (CLP) murine model of prolonged sepsis to show that adoptive transfer of CD34(+) hematopoietic stem-progenitor cells after CLP improves long-term survival by 65%. CD34(+) cell transfer corrected the immunosuppression of late sepsis by (i) producing significantly higher levels of proinflammatory mediators upon ex vivo stimulation with the Toll-like receptor 4 (TLR4) agonist lipopolysaccharide, (ii) enhancing phagocytic activity of peritoneal macrophages, and (iii) clearing bacterial peritonitis. Improved immunity by CD34(+) cell transfer decreased inflammatory peritoneal exudate of surviving late-sepsis mice. Cell tracking experiments showed that the transferred CD34(+) cells first appeared in the bone marrow and then homed to the spleen and peritoneum. Because CD34(+) cells did not affect the early-phase hyperinflammatory response, it is likely that the newly incorporated pluripotent CD34(+) cells differentiated into competent immune cells in blood and tissue, thereby reversing or replacing the hypo-responsive endotoxin-tolerant cells that occur and persist after the initiation of early sepsis (Brudecki, Ferguson et al.).

Carpentier, P. A., A. L. Dingman, et al. "Placental TNF-alpha signaling in illness-induced complications of pregnancy." *Am J Pathol* **178**(6): 2802-10.

Maternal infections are implicated in a variety of complications during pregnancy, including pregnancy loss, prematurity, and increased risk of neurodevelopmental disorders in the child. Here, we show in mice that even mild innate immune activation by low-dose lipopolysaccharide in early pregnancy causes hemorrhages in the placenta and increases the risk of pregnancy loss. Surviving fetuses exhibit hypoxia in the brain and impaired fetal neurogenesis. Maternal Toll-like receptor 4 signaling is a critical mediator of this process, and its activation is accompanied by elevated proinflammatory cytokines in the placenta. We evaluated the role of tumor necrosis factor-alpha (TNF-alpha) signaling and show that TNF receptor 1 (TNFR1) is necessary for the illness-induced placental pathology, accompanying fetal hypoxia, and neuroproliferative defects in the fetal brain. We also show that placental TNFR1 in the absence of maternal TNFR1 is sufficient for placental pathology to develop and that a clinically relevant TNF-alpha antagonist prevents placental pathology and fetal loss. Our observations suggest that the placenta is highly sensitive to proinflammatory signaling in early pregnancy and that TNF-alpha is an effective target for preventing illness-related placental defects and related risks to the fetus and fetal brain development (Carpentier, Dingman et al.).

Das, S., A. Haddadi, et al. (2008). "Delivery of rapamycin-loaded nanoparticle down regulates ICAM-1 expression and maintains an immunosuppressive profile in human CD34+ progenitor-derived dendritic cells." *J Biomed Mater Res A* **85**(4): 983-92.

Immune responses of dendritic cells (DCs) can be modulated by delivery of adjuvants to alter their maturation profile. The purpose of this study was to generate DCs from CD34(+) cells of human cord blood and characterize the effects of poly(D,L-lactic-

co-glycolic acid) (PLGA)-nanoparticle encapsulated rapamycin in generating an immunosuppressive DC. Expression of ICAM-1 (intercellular adhesion molecule), a key molecule in DC-T cell interaction was increased in mature DCs in response to lipopolysaccharide (LPS). When rapamycin was encapsulated in the nanoparticle to maintain DCs in the immature state, ICAM-1 expression was down regulated. When delivered in the free form, rapamycin did not alter the expression of ICAM-1. Cytokine arrays exhibited an immunosuppressive profile of various cytokines in response to the nanoparticulate delivery of rapamycin. In addition, RT-PCR data demonstrated the presence of toll like receptor (TLR) 9 transcripts, although our DCs are myeloid in nature. In summary, our study demonstrates that DCs may be rendered immunosuppressive upon delivery of rapamycin-containing nanoparticles (Das, Haddadi et al. 2008).

Franchini, M., E. Monnais, et al. "Insulin resistance and increased lipolysis in bone marrow derived adipocytes stimulated with agonists of Toll-like receptors." *Horm Metab Res* **42**(10): 703-9.

Our objectives were to identify Toll-like receptors (TLRs) in human bone marrow derived adipocytes, to test specific TLR agonists for their ability to induce a proinflammatory response, and to investigate possible metabolic effects after TLR activation, in particular, those associated with insulin resistance and lipolysis. Mesenchymal stem cells were isolated from human bone marrow and differentiated into adipocytes. Total RNA before or after stimulation with agonists specific for TLR was extracted for analysis of expression of TLRs proinflammatory signals and molecules involved in glucose metabolism (IRS-1 and GLUT4). Furthermore, cytokine protein expression was measured from cell lysates. Finally, insulin induced glucose uptake and lipolysis were measured. Human bone marrow-derived adipocytes express TLR1-10. They react to stimulation with specific ligands with expression of inflammatory markers (IL-1beta, IL-6, TNFalpha, IL-8, MCP-1) at the RNA and protein levels. IRS-1 and GLUT4 expression was downregulated after stimulation with the TLR4 and TLR3 specific ligands LPS and poly (I:C), respectively. Insulin-induced glucose uptake was decreased and lipolysis increased. We conclude that adipocytes express TLR 1-10 and react to agonists specific for TLR 1-6. As a consequence proinflammatory cytokine are induced, in particular, IL-6, IL-8, and MCP-1. Since stimulation is followed by decreased insulin-induced glucose uptake and increased lipolysis we conclude that TLRs may be important linking molecules in the generation of insulin resistance in fat tissue (Franchini, Monnais et al.).

Garantzios, S., S. M. Palmer, et al. (2007). "Alloimmune lung injury induced by local innate immune activation through inhaled lipopolysaccharide." *Transplantation* **84**(8): 1012-9.

BACKGROUND: Alloimmune lung injury, characterized by perivascular lymphocytic inflammation, lymphocytic bronchiolitis (LB), and obliterative bronchiolitis (OB), causes substantial morbidity and mortality after lung transplantation and bone marrow transplantation (BMT), but little is known regarding its pathogenesis. We have developed and pursued the hypothesis that local activation of pulmonary innate immunity through toll-like receptor (TLR)-4 is critical to the development of posttransplant alloimmune lung injury. **METHODS:** We developed a fully major histocompatibility complex-mismatched murine BMT model without systemic graft-versus-host disease, and challenged mice with aerosolized lipopolysaccharide (LPS), a prototypic TLR4 agonist, to determine the effect upon pulmonary alloimmune lung injury. **RESULTS:** LPS-exposed allogeneic BMT recipient mice developed histological and biological features of LB and OB, which were not observed in non-LPS-exposed allogeneic controls or syngeneic LPS-exposed mice. LPS-induced lymphocytic lung inflammation was dependent upon intact TLR4 signaling in donor-derived hematopoietic cells but not recipient structural lung cells, demonstrating a distinct function for TLR4 on hematopoietic cells in mediating alloimmunity. **CONCLUSIONS:** We demonstrate a

critical role for localized, environmentally induced innate immune activation in promoting alloimmune lung injury. Local inhibition of TLR4 signaling in pulmonary resident hematopoietic cells represents a novel and potentially important therapeutic target to prevent posttransplant rejection (Garantziotis, Palmer et al. 2007).

Liu, J., S. Wang, et al. (2009). "Molecular mechanism of the bifunctional role of lipopolysaccharide in osteoclastogenesis." *J Biol Chem* **284**(18): 12512-23.

Lipopolysaccharide (LPS), a common bacteria-derived product, has long been recognized as a key factor implicated in periodontal bone loss. However, the precise cellular and molecular mechanisms by which LPS induces bone loss still remains controversial. Here, we show that LPS inhibited osteoclastogenesis from freshly isolated osteoclast precursors but stimulated osteoclast formation from those pretreated with RANKL in vitro in tissue culture dishes, bone slices, and a co-culture system containing osteoblasts, indicating that RANKL-mediated lineage commitment is a prerequisite for LPS-induced osteoclastogenesis. Moreover, the RANKL-mediated lineage commitment is long term, irreversible, and TLR4-dependent. LPS exerts the dual function primarily by modulating the expression of NFATc1, a master regulator of osteoclastogenesis, in that it abolished RANKL-induced NFATc1 expression in freshly isolated osteoclast precursors but stimulated its expression in RANKL-pretreated cells. In addition, LPS prolonged osteoclast survival by activating the Akt, NF-kappaB, and ERK pathways. Our current work has not only unambiguously defined the role of LPS in osteoclastogenesis but also has elucidated the molecular mechanism underlying its complex functions in osteoclast formation and survival, thus laying a foundation for future delineation of the precise mechanism of periodontal bone loss (Liu, Wang et al. 2009).

Kong, L., L. Sun, et al. (2009). "An essential role for RIG-I in toll-like receptor-stimulated phagocytosis." *Cell Host Microbe* **6**(2): 150-61.

Retinoic acid-inducible gene-I (RIG-I) plays an important role in antiviral response by recognizing double-stranded RNA. Here we demonstrate an unanticipated role of RIG-I in Toll-like receptor (TLR)-stimulated phagocytosis. Stimulation with lipopolysaccharide (LPS), a ligand of TLR4, induced the expression of RIG-I in macrophages. Depletion of RIG-I by RNAi or gene targeting inhibited the LPS-induced phagocytosis of bacteria. Cellular processes involved in phagocytosis, such as small GTPase Cdc42/Rac1 activation, actin polymerization, and actin-regulator Arp2/3 recruitment, were also impaired in RIG-I-deficient macrophages activated by LPS. Moreover, RIG-I(-/-) mice were found to be more susceptible to infection with *Escherichia coli* as compared to wild-type mice. Thus, the regulatory functions of RIG-I are strikingly broad, including a role not only in antiviral responses but in antibacterial responses as well (Kong, Sun et al. 2009).

Keene, C. D., R. Chang, et al. (2009). "Protection of hippocampal neurogenesis from toll-like receptor 4-dependent innate immune activation by ablation of prostaglandin E2 receptor subtype EP1 or EP2." *Am J Pathol* **174**(6): 2300-9.

Prostaglandin E2 is one of several eicosanoid products of the cyclooxygenase isozymes and is a key regulator of innate immune responses; it also possesses paracrine effects on mature neurons. The prostaglandin E2 receptor family consists of four subtypes of which EP1 and EP2 are known to be expressed by microglia. Lipopolysaccharide (LPS)-induced innate immune activation leads to the degeneration of intermediate progenitor cells (IPCs) that are destined for neuronal maturation in the hippocampal subgranular zone (SGZ); these cells can be identified by the expression of the transcription factor T-box brain gene 2 (Tbr2). Importantly, depletion of LPS-induced IPCs from the SGZ is suppressed by cyclooxygenase inhibitors. We therefore tested the

hypothesis that either EP1 or EP2 is critical to LPS-induced depletion of Tbr2+ IPCs from the SGZ. Expression of either EP1 or EP2 was necessary for Toll-like receptor 4-dependent innate immune-mediated depletion of these Tbr2+ IPCs in mice. Moreover, EP1 activation was directly toxic to murine adult hippocampal progenitor cells; EP2 was not expressed by these cells. Finally, EP1 modulated the response of murine primary microglia cultures to LPS but in a manner distinct from EP2. These results indicate that prostaglandin E2 signaling via either EP1 or EP2 is largely to completely necessary for Toll-like receptor 4-dependent depletion of IPCs from the SGZ and suggest further pharmacological strategies to protect this important neurogenic niche (Keene, Chang et al. 2009).

Yu, S., H. H. Cho, et al. (2008). "Role of MyD88 in TLR agonist-induced functional alterations of human adipose tissue-derived mesenchymal stem cells." *Mol Cell Biochem* **317**(1-2): 143-50.

Toll-like receptors (TLRs) sense microorganism components and are critical host mediators of inflammation during infection. Recently, TLRs have been reported to be involved in cell proliferation and differentiation. We previously reported that TLR agonists might affect proliferation and differentiation of human adipose tissue-derived mesenchymal stem cells (hASCs). In this study, we sought to determine whether TLR signaling is dependent on MyD88 in hASCs. The hASCs were downregulated using LV-GFP-miR-MyD88, a lentiviral construct inserted siRNA against human MyD88 that significantly inhibited cell proliferation. MyD88 downregulation reduced NF-kappaB activation and enhancement of osteogenic differentiation induced by peptidoglycan (PGN) more significantly than that induced by lipopolysaccharide (LPS). Although LPS- and PGN-induced cytokine secretions were decreased greatly by MyD88 downregulation, IFN-gamma-induced protein-10 (IP10) and IFNbeta expression were enhanced by LPS irrespective of the downregulation of MyD88. These results suggest that TLR signaling is mediated via MyD88-independent pathways as well as MyD88-dependent pathways in hASCs and that MyD88 contributes to the regulation of cell proliferation and differentiation in hASCs (Yu, Cho et al. 2008).

Turer, E. E., R. M. Tavares, et al. (2008). "Homeostatic MyD88-dependent signals cause lethal inflammation in the absence of A20." *J Exp Med* **205**(2): 451-64.

Toll-like receptors (TLRs) on host cells are chronically engaged by microbial ligands during homeostatic conditions. These signals do not cause inflammatory immune responses in unperturbed mice, even though they drive innate and adaptive immune responses when combating microbial infections. A20 is a ubiquitin-modifying enzyme that restricts exogenous TLR-induced signals. We show that MyD88-dependent TLR signals drive the spontaneous T cell and myeloid cell activation, cachexia, and premature lethality seen in A20-deficient mice. We have used broad spectrum antibiotics to demonstrate that these constitutive TLR signals are driven by commensal intestinal flora. A20 restricts TLR signals by restricting ubiquitylation of the E3 ligase tumor necrosis factor receptor-associated factor 6. These results reveal both the severe proinflammatory pathophysiology that can arise from homeostatic TLR signals as well as the critical role of A20 in restricting these signals in vivo. In addition, A20 restricts MyD88-independent TLR signals by inhibiting Toll/interleukin 1 receptor domain-containing adaptor inducing interferon (IFN) beta-dependent nuclear factor kappaB signals but not IFN response factor 3 signaling. These findings provide novel insights into how physiological TLR signals are regulated (Turer, Tavares et al. 2008).

Tissieres, P., I. Dunn-Siegrist, et al. (2008). "Soluble MD-2 is an acute-phase protein and an opsonin for Gram-negative bacteria." *Blood* **111**(4): 2122-31.

Myeloid differentiation factor-2 (MD-2) is a lipopolysaccharide (LPS)-binding protein usually coexpressed with and binding to Toll-like receptor 4 (TLR4), conferring LPS

responsiveness of immune cells. MD-2 is also found as a soluble protein. Soluble MD-2 (sMD-2) levels are markedly elevated in plasma from patients with severe infections, and in other fluids from inflamed tissues. We show that sMD-2 is a type II acute-phase protein. Soluble MD-2 mRNA and protein levels are up-regulated in mouse liver after the induction of an acute-phase response. It is secreted by human hepatocytic cells and up-regulated by interleukin-6. Soluble MD-2 binds to Gram-negative but not Gram-positive bacteria, and sMD-2 secreted by hepatocytic cells is an essential cofactor for the activation of TLR4-expressing cells by Gram-negative bacteria. Soluble MD-2 opsonization of Gram-negative bacteria accelerates and enhances phagocytosis, principally by polymorphonuclear neutrophils. In summary, our results demonstrate that sMD-2 is a newly recognized type II acute-phase reactant, an opsonin for Gram-negative bacteria, and a cofactor essential for the activation of TLR4-expressing cells. This suggests that sMD-2 plays a key role in the host innate immune response to Gram-negative infections (Tissieres, Dunn-Siegrist et al. 2008).

Rozis, G., A. Benlahrech, et al. (2008). "Human Langerhans' cells and dermal-type dendritic cells generated from CD34 stem cells express different toll-like receptors and secrete different cytokines in response to toll-like receptor ligands." *Immunology* **124**(3): 329-38.

Langerhans' cells (LC) and dermal dendritic cells (dDC) are located in the superficial and deeper layers of the skin respectively and represent the main dendritic cell (DC) populations of the skin. LC-like and dDC-like DC can be generated from CD34 stem cells and this system is widely used as a model for investigating these cells and in therapeutic vaccination. Here we report toll-like receptor (TLR) expression in human LC and dDC derived from CD34 stem cells. In vitro-generated DC expressed TLR-1, 2, 4, 6, 8 and 10. LC, but not dDC, expressed TLR-5, whereas only dDC expressed TLR-3. Maturation of LC was mediated by TLR-2, 4 and 5 ligands, but not by a TLR-3 ligand. dDC maturation was induced by TLR-3 and -4, but not with TLR-5 ligand and only weakly by a TLR-2 ligand. Stimulated LC secreted interleukin (IL)-1beta, low levels of tumour necrosis factor-alpha (TNF-alpha) and IL-8, but not IL-6 or IL-10. dDC secreted TNF-alpha, IL-6, IL-8 and IL-10, but little IL-1beta. IL-12p70 was not produced by ligand-stimulated dDC or LC, but was secreted by monocyte-derived DC (mdDC) stimulated with lipopolysaccharide (LPS). (Rozis, Benlahrech et al. 2008).

Mo, I. F., K. H. Yip, et al. (2008). "Prolonged exposure to bacterial toxins downregulated expression of toll-like receptors in mesenchymal stromal cell-derived osteoprogenitors." *BMC Cell Biol* **9**: 52.

BACKGROUND: Human mesenchymal stromal cells (MSCs, also known as mesenchymal stem cells) are multipotent cells with potential therapeutic value. Owing to their osteogenic capability, MSCs may be clinically applied for facilitating osseointegration in dental implants or orthopedic repair of bony defect. However, whether wound infection or oral microflora may interfere with the growth and osteogenic differentiation of human MSCs remains unknown. This study investigated whether proliferation and osteogenic differentiation of MSCs would be affected by potent gram-positive and gram-negative derived bacterial toxins commonly found in human settings. **RESULTS:** We selected lipopolysaccharide (LPS) from *Escherichia coli* and lipoteichoic acid (LTA) from *Streptococcus pyogenes* as our toxins of choice. Our findings showed both LPS and LTA did not affect MSC proliferation, but prolonged LPS challenge upregulated the osteogenic differentiation of MSCs, as assessed by alkaline phosphatase activity and calcium deposition. Because toll-like receptors (TLRs), in particularly TLR4 and TLR2, are important for the cellular responsiveness to LPS and LTA respectively, we evaluated their expression profiles serially from MSCs to osteoblasts by quantitative PCR. We found that during osteogenic differentiation, MSC-derived osteoprogenitors gradually expressed

TLR2 and TLR4 by Day 12. But under prolonged incubation with LPS, MSC-derived osteoprogenitors had reduced TLR2 and TLR4 gene expression. This peculiar response to LPS suggests a possible adaptive mechanism when MSCs are subjected to continuous exposure with bacteria. **CONCLUSION:** In conclusion, our findings support the potential of using human MSCs as a biological graft, even under a bacterial toxin-rich environment (Mo, Yip et al. 2008).

Komine-Aizawa, S., H. Majima, et al. (2008). "Stimuli through Toll-like receptor (TLR) 3 and 9 affect human chorionic gonadotropin (hCG) production in a choriocarcinoma cell line." *J Obstet Gynaecol Res* **34**(2): 144-51.

AIM: Toll-like receptors (TLR) are expressed mostly on mucosal innate immune cells which recognize microorganism-associated structures, the so called pathogen-associated molecular patterns (PAMP). Recently, possible roles of TLR in placental tissue, especially their role in protecting the conceptus from maternal infection, have been discussed, but the physiopathological roles of TLR in placental functions have yet to be examined. **METHODS:** The choriocarcinoma cell line BeWo was used as a model of trophoblast stem cells. First, the expression of TLR 1-9 on BeWo cells was investigated using reverse transcriptase-polymerase chain reaction. Then, the cells were treated with TLR 1-9 agonists, using the human TLR 1-9 agonist kit (Pam3CKK4, HKLM, Poly (I:C), *Escherichia coli* K12 lipopolysaccharide (LPS), *Salmonella typhimurium* flagellin, FSL1, imiquimod, ssRNA 40, and ODN 2006). The concentration of human chorionic gonadotropin (hCG) in the cell culture supernatants was examined on ELISA. **RESULTS:** BeWo cells constitutively expressed TLR 1-9 mRNA in the presence or absence of forskolin. The TLR 3 agonist, Poly(I:C), and the TLR 9 agonist, CpG-DNA (ODN2006), upregulated hCG production but had minimal effects in the absence of forskolin. Stimulation with the other TLR agonists produced no remarkable increase, whether in the presence or absence of forskolin. **CONCLUSIONS:** TLR 3 and TLR 9 agonists upregulated hCG production in BeWo cells. The present observations suggest alternative roles of TLR signaling in the control of placental functions and blastocyst-endometrial dialogue, as well as in pathophysiology, including for infertility and ectopic pregnancy (Komine-Aizawa, Majima et al. 2008).

Jones, L. A., J. P. Anthony, et al. (2008). "Toll-like receptor-4-mediated macrophage activation is differentially regulated by progesterone via the glucocorticoid and progesterone receptors." *Immunology* **125**(1): 59-69.

Macrophage function has been demonstrated to be subject to modulation by progesterone. However, as this steroid hormone can act through the glucocorticoid receptor as well as the progesterone receptor, the mechanism of action has not been precisely characterized. To determine the mode of action, we compared the ability of progesterone, norgestrel (a synthetic progesterone-receptor-specific agonist) and dexamethasone (a synthetic glucocorticoid receptor agonist) to modulate macrophage function following stimulation of the Toll-like receptor-4 (TLR-4) ligand lipopolysaccharide (LPS). The results demonstrate that following stimulation of TLR-4 with LPS and cotreatment with either progesterone or dexamethasone, but not norgestrel, there is a significant reduction in nitric oxide (NO) production, indicating that this progesterone-mediated effect is through ligation of the glucocorticoid receptor. In contrast, LPS-induced interleukin-12 (IL-12) production could be downregulated by all three steroids, indicating that ligation by progesterone of either the glucocorticoid or the progesterone receptors or both could mediate this effect. While progesterone downmodulated NO-mediated killing of *Leishmania donovani* by activated macrophages in vitro, most probably via the glucocorticoid receptor, it had little effect on *Toxoplasma gondii* growth in these cells. This would suggest that progesterone-mediated increased susceptibility to *T. gondii* during pregnancy is more likely to be related to the ability of the hormone to downregulate IL-12 production and a type-1 response utilizing

the progesterone as well as the glucocorticoid receptors (Jones, Anthony et al. 2008).

Turrin, N. P., M. M. Plante, et al. (2007). "Irradiation does not compromise or exacerbate the innate immune response in the brains of mice that were transplanted with bone marrow stem cells." *Stem Cells* 25(12): 3165-72.

Microglia and invading macrophages play key roles in the brain immune response. The contributions of these two populations of cells in health and diseases have yet to be clearly established. The use of chimeric mice receiving bone marrow-derived stem cell grafts from green fluorescent protein (GFP)-expressing mice has provided an invaluable tool to distinguish between local and blood-derived monocytic populations. The validity of the method is questioned because of the possible immune alterations caused by the irradiation of the recipient mouse. In this experiment, we compared the brain expression of innate immune markers Toll-like receptor 2, interleukin-1 beta, tumor necrosis factor-alpha, and monocyte chemoattractant protein-1 in C57BL/6, GFP, and chimeric mice following an intracerebral injection of lipopolysaccharide. The endotoxin caused a marked transcriptional activation of all these innate immune genes in microglial cells across the ipsilateral side of injection. The expression patterns and signal intensity were similar in the brains of the three groups of mice. (Turrin, Plante et al. 2007).

Shi, L., X. M. Liu, et al. (2007). "[The effect of lipopolysaccharide on the expression and activity of Toll-like receptor 4 in mesenchymal stem cells]." *Zhonghua Xue Ye Xue Za Zhi* 28(12): 828-31.

OBJECTIVE: To investigate the effect of lipopolysaccharide(LPS) on the expression and activity of Toll-like receptor 4(TLR-4) in mesenchymal stem cells (MSC). **METHODS:** MSCs were harvested from adult rats bone marrow cells by density gradient centrifugation and adhesive culture. The purity of MSC were identified with the cell morphology and osteogenic capacity. The phenotypes were assayed by flow cytometry. Cultured MSCs were treated by LPS with various concentration (1 microg/ml, 10 microg/ml or 100 microg/ml) for 24 hours. The relative expression levels of TLR-4 mRNA were detected by semiquantitative RT-PCR, and costimulatory molecules (CD80, CD86 and MHC-II) expressed on MSC were analyzed by flow cytometry. The levels of TNF-alpha in supernatants were determined by double-antibody sandwich ELISA. **RESULTS:** The expression levels of CD80, CD86, MHC-II, TLR-4 mRNA and TNF-alpha in MSC were (9.56 +/- 0.69)%, (22.03 +/- 2.03)%, (2.51 +/- 0.97)%, relative magnitude (0.61 +/- 0.10), (4.97 +/- 2.98) pg/ml, respectively. After incubation with LPS, MSC expressed higher levels of TLR-4 mRNA and costimulatory molecules, and the levels of TNF-alpha were higher than that in untreated group. Among the various concentration of LPS, 10 microg/ml emerged as the most effective in increasing the levels of TLR-4 mRNA (relative magnitude 1.55 +/- 0.02), costimulatory molecules [CD80 (41.70 +/- 2.92)%, CD86 (59.72 +/- 2.00)%, MHC-II (24.56 +/- 2.19)%] and TNF-alpha [(213.12 +/- 69.08) pg/ml] (P < 0.01). (Shi, Liu et al. 2007).

Maratheftis, C. I., E. Andreakos, et al. (2007). "Toll-like receptor-4 is up-regulated in hematopoietic progenitor cells and contributes to increased apoptosis in myelodysplastic syndromes." *Clin Cancer Res* 13(4): 1154-60.

PURPOSE: To investigate the function and expression of Toll-like receptors (TLR) in bone marrow cells of myelodysplastic syndrome (MDS) patients and to examine their involvement in the apoptotic phenomenon characterizing MDS hematopoiesis. **EXPERIMENTAL DESIGN:** TLR mRNA and protein expression was investigated in bone marrow cell populations of MDS patients and controls. TLR-4 ability to recognize lipopolysaccharide and up-regulate self mRNA and protein expression was examined. Tumor necrosis factor involvement in the constitutive and lipopolysaccharide (LPS)-induced TLR expression

was also evaluated. Possible correlation between TLR-4 overexpression and apoptosis was investigated by simultaneous staining with Annexin V and TLR-4. **RESULTS:** TLR-2 and TLR-4 are expressed in almost all bone marrow cell lineages including megakaryocytes, erythroid cells, myeloid precursors, monocytes, and B lymphocytes and are up-regulated in MDS patients compared with controls. In hematopoietic CD34(+) cells, TLR-4 is also expressed and significantly up-regulated at both the mRNA and protein levels. Treatment with an anti-tumor necrosis factor antibody reduces both constitutive and LPS-induced TLR-4 levels. Increased TLR-4 expression correlates with increased apoptosis as TLR-4 is almost exclusively found in apoptotic bone marrow mononuclear and CD34(+) cells. The addition of the TLR-4 ligand LPS further enhances the apoptosis of these cells. **CONCLUSIONS:** TLR-4 and other TLRs are significantly up-regulated in MDS patients whereas TLR-4 is involved in promoting apoptosis, possibly contributing to MDS cytopenia (Maratheftis, Andreakos et al. 2007).

Ma, Y., G. Krikun, et al. (2007). "Cell type-specific expression and function of toll-like receptors 2 and 4 in human placenta: implications in fetal infection." *Placenta* 28(10): 1024-31.

Placental infection is associated with adverse fetal outcomes. Toll-like receptors (TLRs) are critical regulators of the innate immune response based on their ability to recognize and respond to pathogen-associated molecular patterns expressed by microbes. To date, cell-type specific expression and regulation of TLR function in human term placenta remains largely unelucidated. The goal of the current study was to examine the in vivo and in vitro patterns of TLR expression and function in major cell types of term placenta. Immunohistochemical analysis of terminal and stem villi localized TLR-2, which recognizes peptidoglycan (PG) from Gram-positive bacteria, to endothelial cells and macrophages, and to a lesser extent to syncytiotrophoblast (SCTs) and fibroblasts (FIBs). Staining for TLR-4, the receptor for Gram-negative bacterial lipopolysaccharide (LPS), was most prominent in SCTs and endothelial cells. Results from Western blotting, conventional, and quantitative PCR (qRT-PCR) analyses using protein and mRNA isolated from cultures of SCTs and myofibroblasts (mFIBs) revealed that SCTs expressed TLR-2 and TLR-4, whereas mFIBs expressed only TLR-4. In addition, qRT-PCR showed that LPS treatment increased TLR-2 expression in SCTs, indicating that infection with Gram-negative bacteria may enhance innate immune responses in placenta toward a broad range of microorganisms. In addition, treatment with LPS increased IL-8 levels in both SCTs and mFIBs, whereas PG treatment only stimulated IL-8 levels in SCTs. Our results indicate that there exist cell type-specific patterns of TLR function in placenta which likely regulate innate immune response at the maternal-fetal interface (Ma, Krikun et al. 2007).

Zampetaki, A., Q. Xiao, et al. (2006). "TLR4 expression in mouse embryonic stem cells and in stem cell-derived vascular cells is regulated by epigenetic modifications." *Biochem Biophys Res Commun* 347(1): 89-99.

Embryonic stem (ES) cells and ES cell-derived differentiated cells can be used in tissue regeneration approaches. However, inflammation may pose a major hurdle. To define the inflammatory response of ES and ES cell-derived vascular cells, we exposed these cells to LPS. With the exception of MIF no significant cytokine mRNA levels were observed either at baseline or after stimulation. Further experiments revealed that these cells do not express TLR4. Analysis of the DNA methylation status of the TLR4 upstream region showed increased methylation. Moreover, in vitro methylation suppressed TLR4 promoter activity in reporter gene assays. ChIP assays showed that in this region histones H3 and H4 are hypoacetylated in ES cells. Interestingly, 5-aza-dC or TSA partially relieves this gene repression. Finally, the increased levels of TLR4 observed in ES cells after treatment with 5-aza-dC or TSA confer responsiveness to LPS, as induction of IL-6 and TNF-alpha

mRNA was detected in endotoxin stimulated ES cells (Zampetaki, Xiao et al. 2006).

Steiner, A. A., S. Chakravarty, et al. (2006). "Bacterial lipopolysaccharide fever is initiated via Toll-like receptor 4 on hematopoietic cells." *Blood* **107**(10): 4000-2.

Lipopolysaccharide (LPS), a well-known bacterial pyrogen, is recognized by several receptors, including the Toll-like receptor 4 (TLR4), on various cells. Which of these receptors and cells are linked to fever production is unknown. By constructing 4 mouse chimeras and studying their thermoregulatory responses, we found that all 3 phases of the typical LPS fever depend on TLR4 signaling. The first phase is triggered via the TLR4 on hematopoietic cells. The second and third phases involve TLR4 signaling in both hematopoietic and nonhematopoietic cells (Steiner, Chakravarty et al. 2006).

Nagai, Y., K. P. Garrett, et al. (2006). "Toll-like receptors on hematopoietic progenitor cells stimulate innate immune system replenishment." *Immunity* **24**(6): 801-12.

Toll-like receptors (TLRs) are best known for their ability to recognize microbial or viral components and initiate innate immune responses. We showed here that TLRs and their coreceptors were expressed by multipotential hematopoietic stem cells, whose cell cycle entry was triggered by TLR ligation. TLR expression also extended to some of the early hematopoietic progenitors, although not the progenitor cells dedicated to megakaryocyte and erythroid differentiation. TLR signaling via the Myd88 adaptor protein drove differentiation of myeloid progenitors, bypassing some normal growth and differentiation requirements, and also drove lymphoid progenitors to become dendritic cells. CD14 contributed to the efficiency of lipopolysaccharide (LPS) recognition by stem and progenitor cells, and LPS interacted directly with the TLR4/MD-2 complex on these cells in bone marrow. Thus, the preferential pathogen-mediated stimulation of myeloid differentiation pathways may provide a means for rapid replenishment of the innate immune system during infection (Nagai, Garrett et al. 2006).

Glezer, I., A. Lapointe, et al. (2006). "Innate immunity triggers oligodendrocyte progenitor reactivity and confines damages to brain injuries." *FASEB J* **20**(6): 750-2.

Regarded as a damaging reaction, innate immune response can either improve or worsen brain outcome after injury. Hence, inflammatory molecules might modulate cell susceptibility or healing events. The remyelination that follows brain lesions is dependent on the recruitment of oligodendrocyte progenitor cells (OPCs) and expression of genes controlling differentiation and myelin production, such as Olig1 and Olig2 bHLH transcription factors. We aimed to determine how innate immunity affects these processes. Here we report that lipopolysaccharide (LPS) infusion triggered OPC reactivity. Acute inflammation changed the distribution of Olig1- and Olig2-expressing cells following chemical demyelination, enhanced reappearance of transcription signals linked to remyelination and rapidly cleared myelin debris. Although cells expressing Olig1, Olig2, and proteolipid protein were attracted to demyelinated sites in the course of chronic inflammation, myelin loss was not associated with the effects of inflammation on OPC reactivity. In addition, the beneficial properties of brain immunity are broadened to an aggressive model of injury, wherein LPS through Toll-like receptor 4 (TLR4) reduced surfactant-mediated damage while anti-inflammatory treatment enlarged the lesion. In conclusion, TLR4 activation in microglia is a powerful mechanism for improving repair at the remyelination level and protecting the cerebral tissue in presence of agents with strong cytolytic properties (Glezer, Lapointe et al. 2006).

Hollingsworth, J. W., B. J. Chen, et al. (2005). "The critical role of hematopoietic cells in lipopolysaccharide-induced airway inflammation." *Am J Respir Crit Care Med* **171**(8): 806-13.

Rapid and selective recruitment of neutrophils into the airspace in response to LPS facilitates the clearance of bacterial pathogens. However, neutrophil infiltration can also participate in the development and progression of environmental airway disease. Previous data have revealed that Toll-like receptor 4 (tlr4) is required for neutrophil recruitment to the lung after either inhaled or systemically administered LPS from *Escherichia coli*. Although many cell types express tlr4, endothelial cell expression of tlr4 is specifically required to sequester neutrophils in the lung in response to systemic endotoxin. To identify the cell types requiring tlr4 expression for neutrophil recruitment after inhaled LPS, we generated chimeric mice separately expressing tlr4 on either hematopoietic cells or on structural lung cells. Neutrophil recruitment into the airspace was completely restored in tlr4-deficient mice receiving wild-type bone marrow. (Hollingsworth, Chen et al. 2005).

Hayashi, S., M. Tsuneto, et al. (2004). "Lipopolysaccharide-induced osteoclastogenesis in Src homology 2-domain phosphatase-1-deficient viable motheaten mice." *Endocrinology* **145**(6): 2721-9.

Osteoclasts are hemopoietic cells that participate in bone resorption and remodeling. Receptor activator of nuclear factor-kappaB ligand (RANKL) and macrophage colony-stimulating factor (M-CSF) are critical for development of osteoclasts. The Toll-like receptor (TLR) family shares some of the downstream signaling with RANK. The TLR4 ligand, lipopolysaccharide (LPS), is reported to accelerate bone lysis; however, signaling via TLRs has never been reported to induce osteoclastogenesis without RANKL. In this study we showed that significant numbers of mature osteoclasts were generated from protein tyrosine phosphatase Src homology 2-domain phosphatase-1-defective Hcph(me-v)/Hcph(me-v) (me(v)/me(v)) bone marrow cells in the presence of M-CSF and LPS without addition of RANKL in culture. This M-CSF plus LPS-induced osteoclastogenesis was not inhibited by an anti-TNFalpha antagonistic antibody or by osteoprotegerin, a decoy receptor for RANKL. The replacement of RANKL by TLR ligands only occurred with LPS. Other ligands, a peptidoglycan for TLR2 or an unmethylated CpG oligonucleotide for TLR9, did not support osteoclast generation. The osteoclast precursors as well as RANKL-responsive osteoclast precursors were present in the Kit-positive cell-enriched fraction of bone marrow cells. (Hayashi, Yamada et al. 2003).

Schilling, J. D., S. M. Martin, et al. (2003). "Toll-like receptor 4 on stromal and hematopoietic cells mediates innate resistance to uropathogenic *Escherichia coli*." *Proc Natl Acad Sci U S A* **100**(7): 4203-8.

Innate host defenses at mucosal surfaces are critical in the early stages of many bacterial infections. In addition to cells of the traditional innate immune system, epithelial cells can also produce inflammatory mediators during an infection. However, the role of the epithelium in innate host defense in vivo is unclear. Recent studies have shown that lipopolysaccharide (LPS) recognition is critical for bladder epithelial cells to recognize and respond to *Escherichia coli*. Moreover, the LPS-nonresponsive mouse strain C3HHeJ, which has a mutation in the primary LPS receptor, Toll-like receptor 4 (TLR4), is extremely susceptible to infection with uropathogenic strains of *E. coli*. In this study, a bone marrow transplant approach was used to investigate the specific contributions of the bladder epithelium (and other stromal cells) in the TLR4-mediated innate immune response to the invading *E. coli* pathogen. Mice expressing the mutant TLR4 in the epithelialstromal compartment were not able to mount a protective inflammatory response to control the early infection even when their hematopoietic cells expressed wild-type TLR4. (Schilling, Martin et al. 2003).

Moreillon, P. and P. A. Majcherczyk (2003). "Proinflammatory activity of cell-wall constituents from gram-positive bacteria." *Scand J Infect Dis* **35**(9): 632-41.

Innate immunity reacts to conserved bacterial molecules. The outermost lipopolysaccharide (LPS) of Gram-negative organisms is highly inflammatory. It activates responsive cells via specific CD14 and toll-like receptor-4 (TLR4) surface receptor and co-receptors. Gram-positive bacteria do not contain LPS, but carry surface teichoic acids, lipoteichoic acids and peptidoglycan instead. Among these, the thick peptidoglycan is the most conserved. It also triggers cytokine release via CD14, but uses the TLR2 co-receptor instead of TLR4 used by LPS. Moreover, whole peptidoglycan is 1000-fold less active than LPS in a weight-to-weight ratio. This suggests either that it is not important for inflammation, or that only part of it is reactive while the rest acts as ballast. Biochemical dissection of *Staphylococcus aureus* and *Streptococcus pneumoniae* cell walls indicates that the second assumption is correct. Long, soluble peptidoglycan chains (approximately 125 kDa) are poorly active. Hydrolysing these chains to their minimal unit (2 sugars and a stem peptide) completely abrogates inflammation. Enzymatic dissection of the pneumococcal wall generated a mixture of highly active fragments, constituted of trimeric stem peptides, and poorly active fragments, constituted of simple monomers and dimers or highly polymerized structures. (Moreillon and Majcherczyk 2003).

Jensen, B. A., R. J. Leeman, et al. (2003). "Aryl hydrocarbon receptor (AhR) agonists suppress interleukin-6 expression by bone marrow stromal cells: an immunotoxicology study." *Environ Health* 2(1): 16.

BACKGROUND: Bone marrow stromal cells produce cytokines required for the normal growth and development of all eight hematopoietic cell lineages. Aberrant cytokine production by stromal cells contributes to blood cell dyscrasias. Consequently, factors that alter stromal cell cytokine production may significantly compromise the development of normal blood cells. We have shown that environmental chemicals, such as aromatic hydrocarbon receptor (AhR) agonists, suppress B lymphopoiesis by modulating bone marrow stromal cell function. Here, we extend these studies to evaluate the potential for two prototypic AhR agonists, 7,12-dimethylbenz [a]anthracene (DMBA) and 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), to alter stromal cell cytokine responses. **METHODS:** Bone marrow stromal cells were treated with AhR agonists and bacterial lipopolysaccharide (LPS) to mimic innate inflammatory cytokine responses and to study the effects of AhR ligands on those responses. Steady state cytokine RNA levels were screened by RNase protection assays (RPA) and quantified by real-time PCR. Cytokine (IL-6) protein production was measured by ELISA. NF-kappaB EMSAs were used to study IL-6 transcriptional regulation. **RESULTS:** RPAs indicated that AhR+ bone marrow stromal cells consistently up-regulated genes encoding IL-6 and LIF in response to LPS, presumably through activation of Toll-like receptor 4. Pre-treatment with low doses of DMBA or TCDD selectively abrogated IL-6 gene induction but had no effect on LIF mRNA. Real-time-PCR indicated a significant inhibition of IL-6 mRNA by AhR ligands within 1 hour of LPS challenge which was reflected in a profound down-regulation of IL-6 protein induction, with DMBA and TCDD suppressing IL-6 levels as much as 65% and 88%, respectively. This potent inhibitory effect persisted for at least 72 hours. EMSAs measuring NF-kappaB binding to IL-6 promoter sequences, an event known to induce IL-6 transcription, indicated a significant decrease in the LPS-mediated induction of DNA-binding RelA/p50 and c-Rel/p50 heterodimers in the presence of DMBA. (Jensen, Leeman et al. 2003).

Hayashi, S., T. Yamada, et al. (2003). "Distinct osteoclast precursors in the bone marrow and extramedullary organs characterized by responsiveness to Toll-like receptor ligands and TNF-alpha." *J Immunol* 171(10): 5130-9.

Osteoclasts are derived from hemopoietic stem cells and play critical roles in bone resorption and remodeling. Multinucleated osteoclasts are attached tightly to bone matrix, whereas precursor cells with the potential to differentiate into osteoclasts in culture are widely distributed. In this study, we

assessed the characteristics of osteoclast precursors in bone marrow (BM) and in extramedullary organs as indicated by their responsiveness to ligands for Toll-like receptors (TLRs) and to TNF-alpha. Development of osteoclasts from precursor cells in the BM was inhibited by CpG oligonucleotides, a ligand for TLR9, but not by LPS, a ligand for TLR4. BM osteoclasts were induced by TNF-alpha as well as receptor activator of NF-kappaB ligand in the presence of M-CSF. Splenic osteoclast precursors, even in osteoclast-deficient osteopetrotic mice, differentiated into mature osteoclasts following exposure to TNF-alpha or receptor activator of NF-kappaB ligand. However, splenic osteoclastogenesis was inhibited by both LPS and CpG. Osteoclastogenesis from peritoneal precursors was inhibited by not only these TLR ligands but also TNF-alpha. The effects of peptidoglycan, a ligand for TLR2, were similar to those of LPS. BM cells precultured with M-CSF were characterized with intermediate characteristics between those of splenic and peritoneal cavity precursors. Taken together, these findings demonstrate that osteoclast precursors are not identical in the tissues examined. To address the question of why mature osteoclasts occur only in association with bone, we may characterize not only the microenvironment for osteoclastogenesis, but also the osteoclast precursor itself in intramedullary and extramedullary tissues (Hayashi, Yamada et al. 2003).

Sweet, M. J., C. C. Campbell, et al. (2002). "Colony-stimulating factor-1 suppresses responses to CpG DNA and expression of toll-like receptor 9 but enhances responses to lipopolysaccharide in murine macrophages." *J Immunol* 168(1): 392-9.

During bacterial infections, the balance between resolution of infection and development of sepsis is dependent upon the macrophage response to bacterial products. We show that priming of murine bone marrow-derived macrophages (BMMs) with CSF-1 differentially regulates the response to two such stimuli, LPS and immunostimulatory (CpG) DNA. CSF-1 pretreatment enhanced IL-6, IL-12, and TNF-alpha production in response to LPS but suppressed the same response to CpG DNA. CSF-1 also regulated cytokine gene expression in response to CpG DNA and LPS; CpG DNA-induced IL-12 p40, IL-12 p35, and TNF-alpha mRNAs were all suppressed by CSF-1 pretreatment. CSF-1 pretreatment enhanced LPS-induced IL-12 p40 mRNA but not TNF-alpha and IL-12 p35 mRNAs, suggesting that part of the priming effect is posttranscriptional. CSF-1 pretreatment also suppressed CpG DNA-induced nuclear translocation of NF-kappaB and phosphorylation of the mitogen-activated protein kinases p38 and extracellular signal-related kinases-1/2 in BMMs, indicating that early events in CpG DNA signaling were regulated by CSF-1. Expression of Toll-like receptor (TLR)9, which is necessary for responses to CpG DNA, was markedly suppressed by CSF-1 in both BMMs and thioglycolate-elicited peritoneal macrophages. (Sweet, Campbell et al. 2002).

Lehnardt, S., C. Lachance, et al. (2002). "The toll-like receptor TLR4 is necessary for lipopolysaccharide-induced oligodendrocyte injury in the CNS." *J Neurosci* 22(7): 2478-86.

The immediate or innate immune response is the first line of defense against diverse microbial pathogens and requires the expression of recently discovered toll-like receptors (TLRs). TLR4 serves as a specific receptor for lipopolysaccharide (LPS) and is localized on the surface of a subset of mammalian cells. Although innate immunity is a necessary host defense against microbial pathogens, the consequences of its activation in the CNS can be deleterious, as we show here in a developing neural model. We examined the major non-neuronal cell types in the CNS for expression of TLR4 and found that microglia expressed high levels, whereas astrocytes and oligodendrocytes expressed none. Consistent with TLR4 expression solely in microglia, we show that microglia are the only CNS glial cells that bind fluorescently tagged lipopolysaccharide. Lipopolysaccharide led to extensive oligodendrocyte death in culture only under conditions in which microglia were present. To determine whether TLR4 is necessary

for lipopolysaccharide-induced oligodendrocyte death in mixed glial cultures, we studied cultures (Lehnardt, Lachance et al. 2002).

Kadowaki, N., S. Ho, et al. (2001). "Subsets of human dendritic cell precursors express different toll-like receptors and respond to different microbial antigens." *J Exp Med* 194(6): 863-9.

Toll-like receptors (TLRs) are ancient microbial pattern recognition receptors highly conserved from *Drosophila* to humans. To investigate if subsets of human dendritic cell precursors (pre-DC), including monocytes (pre-DC1), plasmacytoid DC precursors (pre-DC2), and CD11c(+) immature DCs (imDCs) are developed to recognize different microbes or microbial antigens, we studied their TLR expression and responses to microbial antigens. We demonstrate that whereas monocytes preferentially express TLR 1, 2, 4, 5, and 8, plasmacytoid pre-DC strongly express TLR 7 and 9. In accordance with these TLR expression profiles, monocytes respond to the known microbial ligands for TLR2 (peptidoglycan [PGN], lipoteichoic acid) and TLR4 (lipopolysaccharide), by producing tumor necrosis factor (TNF)-alpha and interleukin (IL)-6. In contrast, plasmacytoid pre-DCs only respond to the microbial TLR9-ligand, CpG-ODNs (oligodeoxynucleotides [ODNs] containing unmethylated CpG motifs), by producing IFN-alpha. CD11c(+) imDCs preferentially express TLR 1, 2, and 3 and respond to TLR 2-ligand PGN by producing large amounts of TNF-alpha, and to viral double-stranded RNA-like molecule poly I:C, by producing IFN-alpha and IL-12. The expression of distinct sets of TLRs and the corresponding difference in reactivity to microbial molecules among subsets of pre-DCs and imDCs support the concept that they have developed through distinct evolutionary pathways to recognize different microbial antigens (Kadowaki, Ho et al. 2001).

Hayashi, S., T. Yamada, et al. (2003). "Distinct osteoclast precursors in the bone marrow and extramedullary organs characterized by responsiveness to Toll-like receptor ligands and TNF-alpha." *J Immunol* 171(10): 5130-9.

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shown that environmental chemicals, such as aromatic hydrocarbon receptor (AhR) agonists, suppress B lymphopoiesis by modulating bone marrow stromal cell function. Here, we extend these studies to evaluate the potential for two prototypic AhR agonists, 7,12-dimethylbenz [a]anthracene (DMBA) and 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), to alter stromal cell cytokine responses. **METHODS:** Bone marrow stromal cells were treated with AhR agonists and bacterial lipopolysaccharide (LPS) to mimic innate inflammatory cytokine responses and to study the effects of AhR ligands on those responses.

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Toll-like receptors (TLRs) are ancient microbial pattern recognition receptors highly conserved from *Drosophila* to humans. To investigate if subsets of human dendritic cell precursors (pre-DC), including monocytes (pre-DC1), plasmacytoid DC precursors (pre-DC2), and CD11c(+) immature DCs (imDCs) are developed to recognize different microbes or microbial antigens, we studied their TLR expression and responses to microbial antigens. We demonstrate that whereas monocytes preferentially express TLR 1, 2, 4, 5, and 8, plasmacytoid pre-DC strongly express TLR 7 and 9. In accordance with these TLR expression profiles, monocytes respond to the known microbial ligands for TLR2 (peptidoglycan [PGN], lipoteichoic acid) and TLR4 (lipopolysaccharide), by producing tumor necrosis factor (TNF)-alpha and interleukin (IL)-6. In contrast, plasmacytoid pre-DCs only respond to the microbial TLR9-ligand, CpG-ODNs (oligodeoxynucleotides [ODNs] containing unmethylated CpG motifs), by producing IFN-alpha. CD11c(+) imDCs preferentially express TLR 1, 2, and 3 and respond to TLR 2-ligand PGN by producing large amounts of TNF-alpha, and to viral double-stranded RNA-like molecule poly I:C, by producing IFN-alpha and IL-12.

Lehnardt, S., C. Lachance, et al. (2002). "The toll-like receptor TLR4 is necessary for lipopolysaccharide-induced oligodendrocyte injury in the CNS." *J Neurosci* 22(7): 2478-86.

The immediate or innate immune response is the first line of defense against diverse microbial pathogens and requires the expression of recently discovered toll-like receptors (TLRs). TLR4 serves as a specific receptor for lipopolysaccharide (LPS) and is localized on the surface of a subset of mammalian cells. Although innate immunity is a necessary host defense against microbial pathogens, the consequences of its activation in the CNS can be deleterious, as we show here in a developing neural model. We examined the major non-neuronal cell types in the CNS for expression of TLR4 and found that microglia expressed high levels, whereas astrocytes and oligodendrocytes expressed none. Consistent with TLR4 expression solely in microglia, we show that microglia are the only CNS glial cells that bind fluorescently tagged lipopolysaccharide. Lipopolysaccharide led to extensive oligodendrocyte death in culture only under conditions in which microglia were present. To determine whether TLR4 is necessary for lipopolysaccharide-induced oligodendrocyte death in mixed glial cultures, we studied cultures generated from mice bearing a loss-of-function mutation in the *tlr4* gene.

Moreillon, P. and P. A. Majcherczyk (2003). "Proinflammatory activity of cell-wall constituents from gram-positive bacteria." *Scand J Infect Dis* 35(9): 632-41.

Innate immunity reacts to conserved bacterial molecules. The outermost lipopolysaccharide (LPS) of Gram-negative organisms is highly inflammatory. It activates responsive cells via specific CD14 and toll-like receptor-4 (TLR4) surface receptor and

co-receptors. Gram-positive bacteria do not contain LPS, but carry surface teichoic acids, lipoteichoic acids and peptidoglycan instead. Among these, the thick peptidoglycan is the most conserved. It also triggers cytokine release via CD14, but uses the TLR2 co-receptor instead of TLR4 used by LPS. Moreover, whole peptidoglycan is 1000-fold less active than LPS in a weight-to-weight ratio. This suggests either that it is not important for inflammation, or that only part of it is reactive while the rest acts as ballast. Biochemical dissection of *Staphylococcus aureus* and *Streptococcus pneumoniae* cell walls indicates that the second assumption is correct. Long, soluble peptidoglycan chains (approximately 125 kDa) are poorly active. Hydrolysing these chains to their minimal unit (2 sugars and a stem peptide) completely abrogates inflammation. Enzymatic dissection of the pneumococcal wall generated a mixture of highly active fragments, constituted of trimeric stem peptides, and poorly active fragments, constituted of simple monomers and dimers or highly polymerized structures.

Schilling, J. D., S. M. Martin, et al. (2003). "Toll-like receptor 4 on stromal and hematopoietic cells mediates innate resistance to uropathogenic *Escherichia coli*." *Proc Natl Acad Sci U S A* 100(7): 4203-8.

Innate host defenses at mucosal surfaces are critical in the early stages of many bacterial infections. In addition to cells of the traditional innate immune system, epithelial cells can also produce inflammatory mediators during an infection. However, the role of the epithelium in innate host defense *in vivo* is unclear. Recent studies have shown that lipopolysaccharide (LPS) recognition is critical for bladder epithelial cells to recognize and respond to *Escherichia coli*. Moreover, the LPS-nonresponsive mouse strain C3HHeJ, which has a mutation in the primary LPS receptor, Toll-like receptor 4 (TLR4), is extremely susceptible to infection with uropathogenic strains of *E. coli*. In this study, a bone marrow transplant approach was used to investigate the specific contributions of the bladder epithelium (and other stromal cells) in the TLR4-mediated innate immune response to the invading *E. coli* pathogen.

Skripuletz, T., E. Miller, et al. "Lipopolysaccharide delays demyelination and promotes oligodendrocyte precursor proliferation in the central nervous system." *Brain Behav Immun* 25(8): 1592-606.

Systemic infection can influence the course in many diseases of the central nervous system (CNS) such as multiple sclerosis (MS), yet the relationship between infection outside the CNS and potential damage and/or protection within the CNS is still not understood. Activation of microglia is a characteristic feature of most CNS autoimmune disorders, including MS, and both protective and degenerative functions of microglia have been proposed. Hence, we analyzed the effects of a systemic inflammatory reaction induced by peripheral treatment with lipopolysaccharide (LPS) on microglial reaction and cuprizone induced de- and remyelination. We found that LPS administration delayed demyelination, which was linked with inhibition of microglial proliferation and reduced numbers of activated microglia. The phenotype of microglia changed as an increase of Toll-like receptor 4 was found. During remyelination, LPS treatment delayed the onset of myelin protein re-expression, but later there was a beneficial effect via an increase of proliferating oligodendrocyte precursor cells (OPC) and mature oligodendrocytes.

Steiner, A. A., S. Chakravarty, et al. (2006). "Bacterial lipopolysaccharide fever is initiated via Toll-like receptor 4 on hematopoietic cells." *Blood* 107(10): 4000-2.

Lipopolysaccharide (LPS), a well-known bacterial pyrogen, is recognized by several receptors, including the Toll-like receptor 4 (TLR4), on various cells. Which of these receptors and cells are linked to fever production is unknown. By constructing 4 mouse chimeras and studying their thermoregulatory responses, we found that all 3 phases of the typical LPS fever depend on TLR4

signaling. The first phase is triggered via the TLR4 on hematopoietic cells. The second and third phases involve TLR4 signaling in both hematopoietic and nonhematopoietic cells (Steiner, Chakravarty et al. 2006).

Sweet, M. J., C. C. Campbell, et al. (2002). "Colony-stimulating factor-1 suppresses responses to CpG DNA and expression of toll-like receptor 9 but enhances responses to lipopolysaccharide in murine macrophages." *J Immunol* 168(1): 392-9.

During bacterial infections, the balance between resolution of infection and development of sepsis is dependent upon the macrophage response to bacterial products. We show that priming of murine bone marrow-derived macrophages (BMMs) with CSF-1 differentially regulates the response to two such stimuli, LPS and immunostimulatory (CpG) DNA. CSF-1 pretreatment enhanced IL-6, IL-12, and TNF-alpha production in response to LPS but suppressed the same response to CpG DNA. CSF-1 also regulated cytokine gene expression in response to CpG DNA and LPS; CpG DNA-induced IL-12 p40, IL-12 p35, and TNF-alpha mRNAs were all suppressed by CSF-1 pretreatment. CSF-1 pretreatment enhanced LPS-induced IL-12 p40 mRNA but not TNF-alpha and IL-12 p35 mRNAs, suggesting that part of the priming effect is posttranscriptional.

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